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# EEG SYNCHRONIZATION AND BEHAVIORAL SIGNS OF SLEEP FOLLOWING LOW FREQUENCY STIMULATION OF THE BRAIN STEM RETICULAR FORMATION

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## INTRODUCTION

It is well known that transition from wakefulness to sleep is electroencephalographically characterized by the replacement of low voltage fast rhythms with high voltage slow activity. The corresponding terms "desynchronization" and "synchronization" were introduced by Adrian (2, 1) to explain the intrinsic mechanisms of the EEG and are often used today to denote the two types of EEG activity even independently of their original meaning.

Transition from wakefulness and EEG desynchronization to sleep and EEG synchronization has been explained: *a*) by a depression of an activating, EEG-desynchronizing system; *b*) by an increase in the activity of sleep-inducing, EEG-synchronizing structures (see 40 for references). The two hypotheses are not necessarily conflicting (40, 35).

The identification of the principal arousing and EEG-desynchronizing system with the reticular formation of the brain stem is now so well established that it is not necessary to recall here the number of investigations on which it is based (see 46). Our knowledge of the neural structures having sleep-inducing and EEG-synchronizing action is, on the contrary, far from satisfactory.

The pioneer behavioral studies of Hess (24, 25, 26, 27, 28, 29) and the classical EEG observations of Dempsey and Morison (39, 16) showed that physiological sleep and EEG synchronization could be produced by adequate electrical stimulation of certain thalamic nuclei. Both effects were subsequently obtained in one and the same animal by other authors (30, 37, 3, 4, 38), so that the importance of the talamus in the regulation of the sleep-wakefulness rhythm, or at least of the electrical activity of the cerebral cortex, is now generally accepted.

Several experimental observations accumulated in recent years seem to indicate that behavioral signs of sleep (12, 29, 14, 45, 44) and EEG synchronization (48, 18, 14, 7, 31, 33, 43, 42, 49, 34) may be produced also by electrical stimulation of the midbrain tegmentum and central grey substance. Moreover, evidence for the existence of a tonic EEG-synchronizing and possibly sleep-inducing influence arising in the lower brain stem was provided by the demonstration of the persistent EEG and behavioral patterns of wakefulness following complete transection of the neuraxis at midpontine pretrigeminal level (8, 9) and by the EEG asymmetries occurring after unilateral midpontine transection (15).

In view of the well known sleep-inducing (32) and EEG-synchronizing (10, 20, 36, 19, 11) influence of carotid sinus pressoceptors, it has been assumed that at least one of these synchronizing structures could be the bulbar sensory nuclei receiving glossopharingeal and vagal afferentes (35). As a matter of fact, by direct stimulation of the region of the nucleus of the solitary tract in the *éncephale isolé* cat, Magnes, Moruzzi and Pompeiano (35) succeeded in eliciting EEG synchronization. On the other hand, in preliminary research made in normal unanesthetized cats carrying chronically implanted electrodes, we observed that if low rate stimulation pulses were used, synchronized EEG patterns similar to those occurring during sleep could be elicited by the reticular formation of the whole brain stem, at midbrain, pontine and bulbar level (17).

On the whole, the data obtained thus far certainly point to the existence in the brain stem of sleep-inducing and EEG-synchronizing mechanisms. However, a clear and complete picture of the structures responsible for these mechanisms is not available. We thought that the systematic exploration of the brain stem with stimulating electrodes might be useful. Following the results obtained in our preliminary research mentioned above (17), our study was limited to the reticular formation; all districts of this widely distributed structure were carefully explored on intact animals. Particular attention was given to a parallel study of behavioral and EEG phenomena with the aim of determining whether sleep occurred whenever EEG synchronization was elicited.

## METHODS

The experiments were performed in forty unanesthetized intact adult cats. Screw electrodes inserted in the calvarium for EEG recording and needle bipolar electrodes for reticular stimulation were permanently implanted with aseptic precautions under barbiturate anesthesia. The screw electrodes were inserted through a small trephine hole over frontal, temporal and occipital areas of both sides. The subcortical needle electrodes (two paired copper or steel wires insulated except for the extremity, with an interelectrode distance of 1 to 1.5 mm) were oriented with the stereotaxic instrument and fixed to the scalp with dental cement. No more than four subcortical electrodes were used in each cat. The wires from both screw and needle electrodes terminated in a small light socket placed on a leather bag which the cat carried on its back. This socket was connected with recording and stimulating instruments through leads long enough to allow for the animal's complete freedom of movement. Bipolar records were taken on a Galileo 10-channel inkwriting electroencephalograph. Square wave pulses of variable duration, frequency and intensity were employed for the stimulation.

The experimental sessions started at least two days after electrodes were implanted and were repeated several times for a maximum of 20 days. Each session lasted from 2 to 5 hours. As a rule, the reticular formation was stimulated bipolarly by trains of pulses, with a repetition rate of 5 to 10/sec; each stimulating train had a duration of 5 to 10 sec and was more or less regularly repeated every 10-20 sec. To avoid disturbances occasioned by noises in the experimental room, the cat was often placed in a soundproof cage; the experimenter could look at the cat through a small window.

The animals were sacrified by administration of an overdose of barbiturate and the brain fixed by intracarotid injection of 15% formaline solution when the electrodes were still *in situ*. The precise position of the reticular electrodes was then determined by examining the electrode track on serial sections of the brain stem stained with the Nissl and Weil methods.

## RESULTS

1. *EEG synchronization following low frequency stimulation of the reticular formation.* — In confirmation of our preliminary report (17), high voltage, slow waves appeared on the EEG record during low frequency stimulation of the reticular formation of the brain stem.

*The typical pattern of induced EEG synchronization* is illustrated in Fig. 1. The animal was lying in a sleeping attitude with half closed eyelids; the EEG rhythms, however, were predominantly desynchronized (Fig. 1, *A*). The stimulating electrode was located in the left part of the reticular formation of the caudal pons (Fig. 1, *A*). The first train of stimulating pulses did not give clear cut EEG changes (Fig. 1, *A*). The second train of stimuli yielded a short lasting generalized burst of high amplitude 7-8/sec waves (Fig. 1, *B*). During the third train of stimuli the generalized slow waves appeared at the onset of stimulation, reached their largest amplitude in 2-3 sec and disappeared when the stimulation ceased (Fig. 1, *B*). The fourth train of stimulating pulses gave an

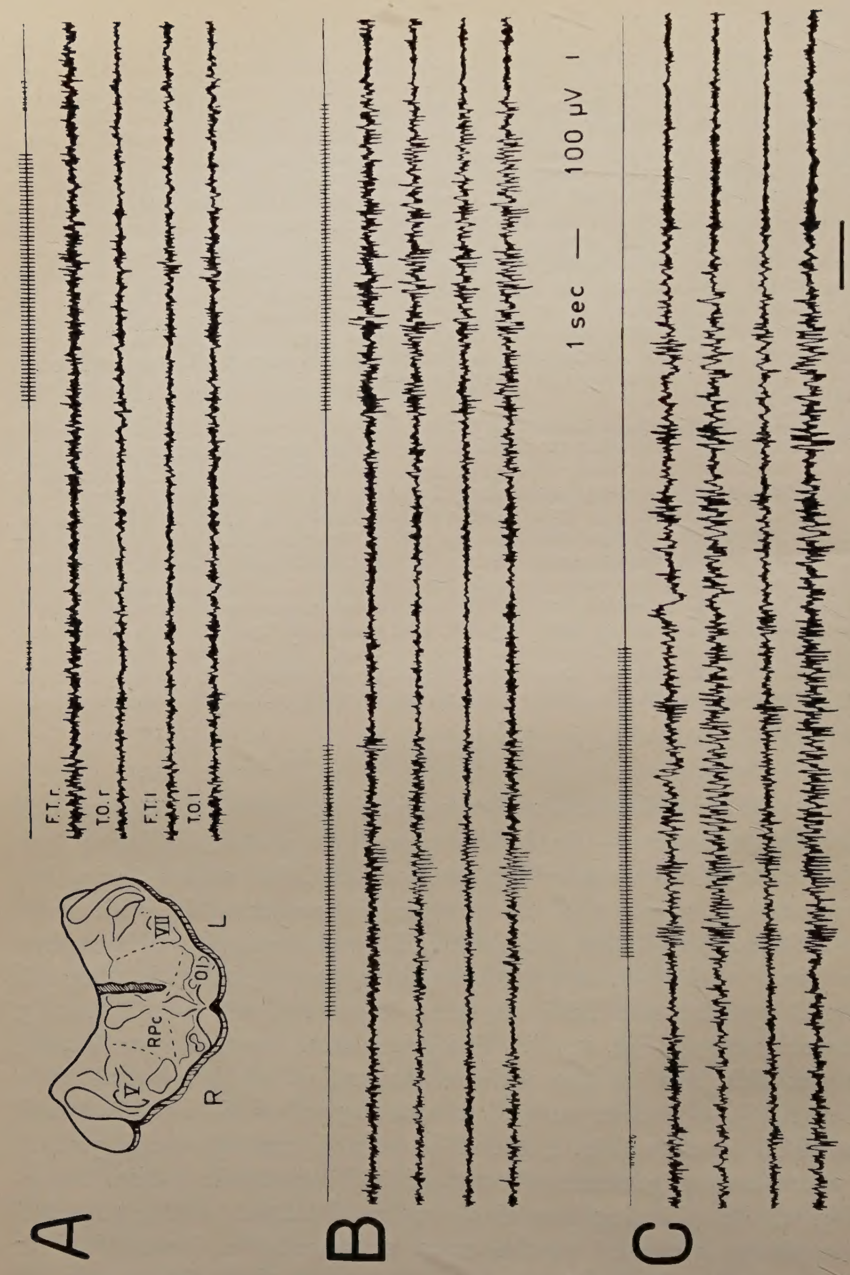

Fig. 1.

EEG response similar to the preceding one; this time, however, the slow high amplitude waves persisted after the end of the stimulation until they were suppressed by a sensory stimulus (Fig. 1, *C*).

This pattern of response was observed several times in the same cat and in most of our animals. However, the EEG effect of low frequency reticular stimulation did not always develop in this way. In some instances the trains of stimulating pulses had to be repeated several times before any EEG synchronization could be observed. On the other hand, it was not infrequently observed that the synchronizing effect could be elicited with the very first train of stimulating pulses. Moreover, synchronization did not always persist after stimulation was terminated. This variability

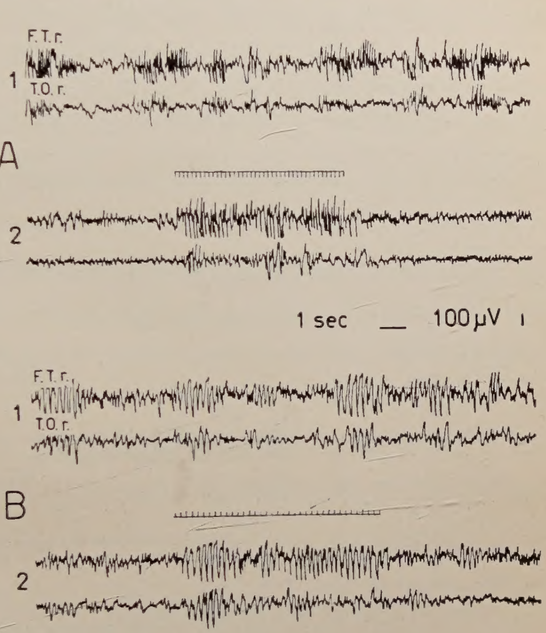

Fig. 2. – *Relation between spontaneous and induced EEG synchronization.*

In *A* and *B* are the bipolar EEG records from two different normal cats. *1*: spontaneous EEG synchronization appearing during drowsiness or sleep. *2*: synchronization induced by low frequency stimulation of the midbrain reticular substance (*A*: 0.1 msec, 7/sec, 3 V; *B*: 0.1 msec, 5/sec, 4 V). Bipolar EEG records from fronto-temporal (F. T.) and temporo-occipital (T. O.) areas of right side (r.).

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Fig. 1. – *EEG synchronizing effects of repeated trains of low frequency electrical pulses applied to the reticular formation.*

Normal cat. The track indicating the exact location of the stimulating electrode (in the nucleus reticularis pontis caudalis: RPc) is represented by the cross-hatched area in the drawing of a traverse section of the brain stem. Continuous bipolar EEG records from right (r.) and left (l.) fronto-temporal (F. T.) and temporo-occipital (T. O.) areas. In this and in the following figures the signals of the electrical stimulation of the reticular substance are placed above the EEG record (in this case 0,2 msec, 7/sec, 4 V pulses were used). A sensory stimulus (whistle) producing EEG activation reation in C is marked by a black line below the EEG records.

of the EEG response to low frequency reticular stimulation might be well understood by taking into consideration possible interference by certain factors to be described below.

In spite of this variability, it seemed nonetheless quite clear that low frequency stimulation of the reticular structures had the following EEG effects: *a*) the production of a train of slow high voltage waves more or less coincident in time with the trains of stimulating pulses; *b*) the development of a continuous synchronization, which persisted even when the reticular stimulation was stopped. Although the induced slow high amplitude waves were quite similar in both cases, the first effect appeared to be directly dependent on the single train of stimulating pulses, while the second appeared to be the result of the cumulative action of several stimulating trains.

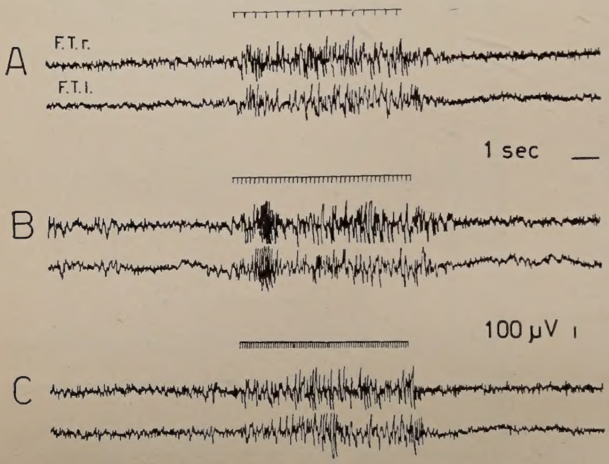

Fig. 3. – *Frequency of reticular stimulation, and configuration and frequency of induced EEG synchronization.*

Normal cat. Bipolar EEG records from frontotemporal (F. T.) areas of both sides (r. and l.). Stimulation of the right bulbar reticular substance with 0.1 msec, 4 V, 3/sec (*A*), 7/sec (*B*) and 12/sec (*C*).

Fig. 2 shows that the patterns of the EEG rhythms induced by low rate reticular stimulation were not uniform in the different animals. As a matter of fact, both the configuration and the frequency of the induced slow waves were related more to the type of the synchronized EEG rhythms appearing spontaneously during drowsiness or sleep (Fig. 2) than to the physical parameters of the reticular stimulation (Fig. 3), or to the anatomical location of the reticular electrodes.

Also the cortical distribution of the induced EEG rhythms was similar to that of the spontaneous synchronization. Thus for instance, in those cases in which the spontaneously occurring high voltage slow waves were prevalent in the posterior or in the anterior regions, those induced by reticular stimulation were also more evident in the same areas. The only difference sometimes observed in the cortical distribution of spontaneous and induced EEG synchronization was that the latter, although always generalized, might be more evident in the hemisphere ipsilateral to the stimulated side.

Finally, sensory stimuli (Fig. 1, *C*; 4, *A*) or high rate stimulation of the reticular formation (Fig. 4, *B*) blocked the high voltage slow waves induced by low rate reticular pulses. The activation of the EEG was as immediate and complete as that produced on the spontaneous synchronous rhythms.

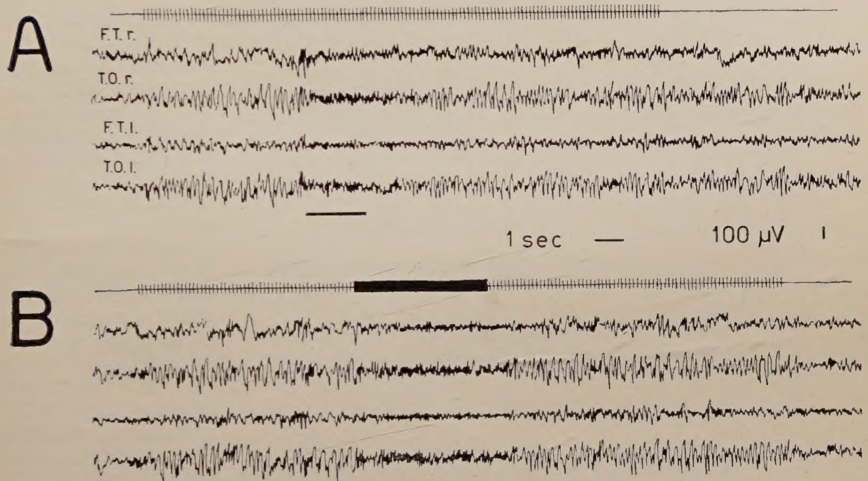

Fig. 4. – *Blocking effect of sensory stimuli and of high frequency reticular stimulation on the induced EEG synchronization.*

Normal cat. Bipolar EEG records from right (r.) and left (l.) frontotemporal (F. T.) and temporo-occipital (T. O.) areas. *A*: a whistle (black line below the EEG records) blocks the synchronization induced by stimulating the pontine reticular substance with 0.2 msec, 7/sec, 4 V. *B*: same effect as in *A* by increasing the frequency of the reticular stimulation to 25/sec.

*The reticular sites which produced the EEG synchronizing effect* were located at all levels of the brain stem, in the midbrain, pons and medulla oblongata. As shown in Fig. 5, stimulating electrodes were situated in the following subdivisions of the reticular formation (see 46): dorsal and ventral tegmentum in the midbrain; nucleus oralis, nucleus tegmenti of Bechterew and nucleus caudalis in the pons; nucleus giganto-cellularis, nucleus parvo-cellularis and nucleus ventralis in the medulla oblongata. EEG-synchronizing effects were elicited by all these reticular districts (black dots in Fig. 5). However, out of a total number of 48 reticular electrodes, 43 gave a good response, while from the remaining 5 no EEG synchronization could be elicited (circles in Fig. 5). Actually, in two instances EEG arousal was produced by low frequency stimulation (Fig. 6, *B*). It may be worth noting: *a*) that 3 of the 5 electrode positions from which EEG synchronization was not induced were located at the peripheral borders of the reticular formation, *i. e.*, very close to the specific sensory pathways (Fig. 5, sections 2 and 3; Fig. 6);

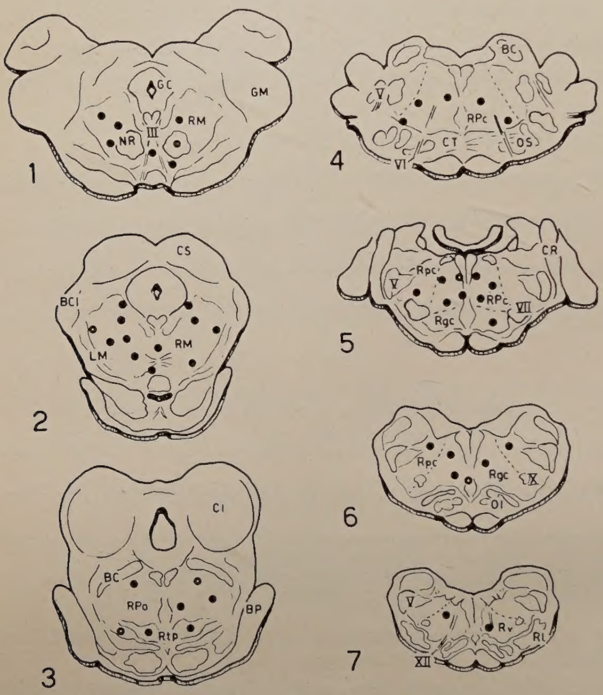

Fig. 5. – *Anatomical location of the reticular sites stimulated in the present research.*

The brain stem of the cat is reproduced in seven drawings of transverse sections. Black dots mark the anatomical location of the tip of the electrodes giving EEG synchronization. Circles mark the sites from which EEG synchronization could not be elicited.

BC: brachium conjuntivum. BCI: brachium colliculi inferioris. BP: brachium pontis. CI: colliculus inferior. CR: corpus restiforme. CS: colliculus superior. CT: corpus trapezoide. GC: substantia grisea centralis. GM: corpus geniculatum mediale. LM: lemniscus medialis. NR: nucleus ruber. OI: oliva inferior. OS: oliva superior. Rgc: nucleus reticularis gigantocellularis. Rl: nucleus reticularis lateralis (of lateral funiculus). RM: substantia reticularis mesencephali. RPc: nucleus reticularis pontis caudalis. Rpc: nucleus reticularis parvocellularis. RPo: nucleus reticularis pontis oralis. Rtp: nucleus reticularis tegmenti pontis (of Bechterew). Rv: nucleus reticularis ventralis. *III*, *V*, *VI*, *VII*, *X* and *XII*: nuclei or fibers of cranial nerves.

*b*) that in the same cats carrying the electrodes from which no EEG synchronization could be obtained, or which gave EEG desynchronization, at least one other reticular electrode gave a good synchronizing effect in the same experimental session (Fig. 6); *c*) that several other electrodes (not mapped in Fig. 5) were placed in extra-reticular structures (as for instance the central periaqueductal grey or the trigeminal sensory nuclei) and EEG synchronization was not induced from them, even though it had been evoked easily in the same animal and in the same experimental session by reticular stimulation.

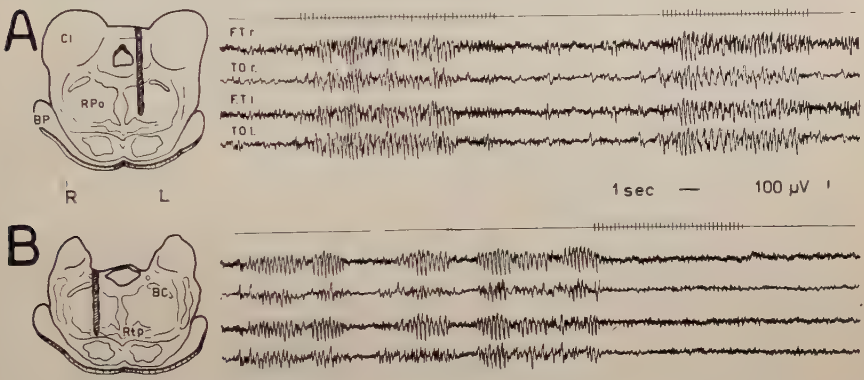

Fig. 6. – *Opposite EEG effects produced by low frequency stimulation of two different reticular sites.*

Normal cat. Bipolar EEG records from right (r.) and left (l.) fronto-temporal (F. T.) and temporo-occipital (T. O.) areas. *A*: 0.1 msec, 5/sec, 5 V to the left nucleus reticularis pontis oralis (RPo) produces EEG synchronization. *B* (2 min after *A*): 0.1 msec, 5/sec, 5 V to the most ventral part of the same reticular nucleus in the right side produces EEG activation.

*Frequency of stimulating pulses and background EEG activity* were the most important elements on which the synchronization induced by reticular stimulation depended.

According to our experience, the most suitable pulse rates for producing synchronization were between 4/sec and 14/sec. Stimulation below 3/sec was generally not followed by any EEG changes, while 20/sec and above always produced a typical EEG arousal reaction (Fig. 4, *B*). Usually, the optimum repetition rate of the stimulating pulses corresponded to that observed during the spontaneous bursts of EEG synchronization. However, as noted above, the frequency of the induced synchronous EEG waves and that of the electric stimulation were not strictly related to each other (Fig. 3).

The fundamental importance of working on a favorable background of EEG activity in order to obtain EEG synchronization by reticular stimulation was apparent from our early experiments (17). As a matter of fact, at the beginning of our study, the establishment of a favorable EEG background proved to be one of the most serious technical problems. Actually, we decided to work on chronic, intact, unanesthetized animals, because we wanted the opportunity to test and compare in the same animal the efficacy of our stimulation on all the possible EEG conditions, from the full synchronization of deep sleep to the complete desynchronization of alertness. It soon became manifest that synchronizing effects could consistently be elicited only when the EEG activity was of the type usually observed in the relaxed waking animal (23), *i. e.*, a low amplitude rhythm with occasional bursts of 5-8/sec high voltage waves (Fig. 7, *B*). No EEG-synchronizing effect could be obtained on a background of complete desynchronization (Fig. 7, *A*) such as that occurring during alertness, nor could it be recognized on a

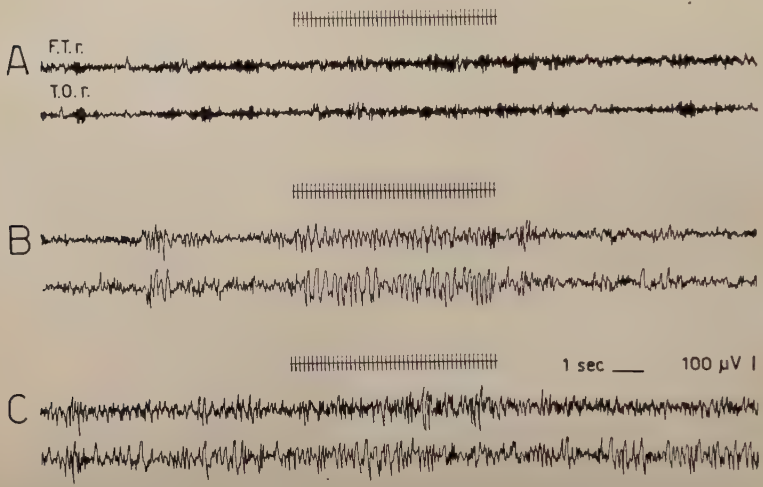

Fig. 7. – *EEG background and synchronizing effect of low frequency reticular stimulation.*

Normal cat. Bipolar EEG records from right (r.) fronto-temporal (F. T.) and temporo-occipital (T. O.) areas. Stimulation of the pontine reticular formation with 0.1 msec, 7/sec, 6 V. Background activity completely desynchronized in *A* (the animal is awake), chiefly desynchronized with some bursts of slow waves in *B* (relaxed wakefulness) and synchronized in *C* (drowsiness). The synchronizing effect is evident only in *B*.

background of continuous synchronization (Fig. 7, *C*) accompanying sleep. The extreme sensitivity of the EEG-synchronizing reticular effect to the background EEG activity may explain the variability in the development of induced synchronization.

*The effect of barbiturates on the EEG-synchronizing reaction to low frequency reticular stimulation* was studied in some of our animals. Once the best parameters of reticular stimulation had been established, a narcotic dose of a slow acting barbiturate (pentobarbital sodium) was injected intraperitoneally. As could be expected on the basis of the observations made on the influence of the EEG background on induced EEG synchronization, soon after the barbiturate injection, when the first spindle bursts appeared on the EEG, reticular synchronizing effects were well manifested; a few minutes thereafter, when the typical EEG synchronized patterns of the barbiturization were fully developed, the synchronizing effects were lost, nor could they be obtained during the deepest stage of the barbiturate narcosis, when the EEG was almost flat with only rare bursts of spindles.

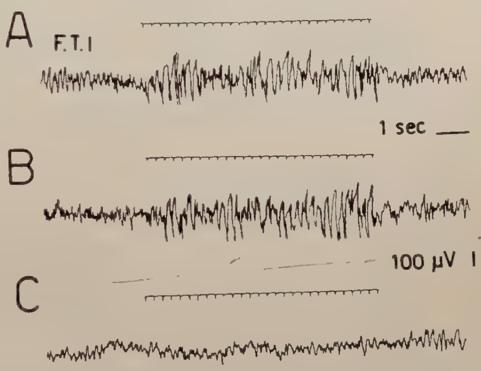

Fig. 8. – *Effects of cervical spinal transection, curarization and destruction of the stimulated tissue on the induced EEG synchronization.*

EEG records from the left fronto-temporal regions (F.T.l.). Stimulation of the left midbrain reticular subtance with 0.1 msec, 5/sec, 3 V. *A*: control. *B* (same animal): 30 min after transection of the spinal cord at Cl (*encéphale isolé* preparation) and 5 min after the i. v. injection of a paralyzing dose of Sincurarina. *C* (same animal): 5 min after electrolytic lesion (2.5 mA for 15 sec). the stimulation of the previously excited structure is without effect.

*Control experiments* were performed to eliminate some possible sources of error in the interpretation of the EEG changes produced by low frequency stimulation of the reticular substance.

In some of our chronic animals which were carrying reticular electrodes yielding good EEG-synchronizing effects upon low frequency stimulation (Fig. 8, *A*), a tracheal cannula was inserted under ether anesthesia, and the spinal cord was severed between the first and the second cervical segment under artificial ventilation (*encéphale isolé* preparation). By continuous intravenous administration of dilute noradrenaline, blood pressure was maintained at the level of 100 mm Hg. When the ether was eliminated – provided that a favourable EEG background of the type described above was present – synchronizing effects were easily elicited as had been the case in the intact animals (Fig. 8, *B*). Thus we eliminated the possible objection that the EEG response to reticular stimulation might be due to changes in blood pressure and respiration. A paralysing dose of *Sincurarina* was then administered intravenously. No modification of the induced synchronization was observed (Fig. 8, *B*). The synchronization, therefore, could not be ascribed to muscular artifacts or to proprioceptive reverberation due to the well known reticular influences on the $\alpha$ and $\gamma$ motoneurons of the spinal cord (see 46 for references). Finally, the nervous tissue surrounding the tip of the stimulating reticular electrode was destroyed with electrolysis, by passing a direct current of 2.5 mA between the two extremities of the electrode for 15 sec. When the lesion was made, the low frequency stimulation completely lost its efficacy (Fig. 8, *C*) and EEG synchronization could not be elicited even by increasing the intensity of stimulation. This would indicate that the synchronizing effects were not due to spread of the stimulating current to neighboring structures.

2. *Behavioral signs of sleep following low frequency stimulation of the reticular formation.* — As we stressed above, the EEG effects of low frequency reticular stimulation were to a great extent dependent on the degree of existing activation or synchronization of the electrocortical activity (see pag. 10). In our intact unanesthetized animals the spontaneous EEG activity could be easily related to behavior, and the conclusion was reached that the development of the EEG synchronization by reticular stimulation was dependent on the degree of alertness or sleep at the moment of stimulation.

Attention was then paid to the possible changes of animal behavior following low frequency reticular stimulation capable of inducing EEG slow rhythms. The observations made during one typical experiment are given in the following abstract of protocol.

Cat number 10 – Session n. 4. Bipolar stimulation of the bulbar reticular substance (nucleus reticularis parvocellularis) with pulse trains of 5 sec repeated every 10 sec; pulse duration 0.1 msec; repetition rate 7/sec; intensity 5 V. The cat was good natured and co-operative. At the beginning of the experiment the spontaneous EEG activity was predominantly desynchronized.

" Initially, short bursts of synchronization appear on the EEG one or two seconds after the beginning of each train of stimulating pulses and disappear at the end of it. By repeating the stimulation at regular intervals, the induced synchronization becomes more and more evident and outlasts each stimulating train. Finally, 2 min after the first stimulation, continuous synchronization is present on the EEG even when the stimulation is stopped completely. As the EEG-synchronizing effects of the reticular stimulation progressively increase, the animal – which initially was lying in relaxed waking attitude, with lids completely opened and pupils slightly dilated – reclines the head while the lids become almost closed and the pupils very narrow. This sleeping attitude is maintained for several minutes after the last stimulation it is finally modified by a strong whistle which produces a typical behavioral and EEG arousal reaction ".

These results were obtained several times in this same animal and could be reproduced in many other experiments independently of the location of the stimulated reticular site. Therefore, we had the impression that a tendency to sleep followed the low frequency stimulation of the reticular substance yielding EEG synchronization.

However, we have to emphasize that results of the type just reported were by no means attained constantly. In describing the EEG effects of low frequency reticular stimulation we said that in several cases synchronized rhythms did not develop in the intervals between the trains of stimulating pulses, nor did they persist when the stimulation was stopped completely. Likewise, in several instances – in spite of the repeated attempts made – we observed no behavioral changes suggesting that the animal was going to sleep or was actually sleeping. As a matter of fact, the two phenomena, an EEG synchronization developing between and after the trains of electrical pulses applied to the reticular formation and the appearance of behavioral signs of sleep were strictly related. EEG synchronization outlasting the reticular stimulation was always associated with a sleeping attitude and, *viceversa*, behavioral manifestations of sleep were never elicited by reticular stimuli without EEG synchronization [1]. No clear cut indication of behavioral sleep was obtained when the appearance of short lasting bursts of synchronous waves coincided with, but did not outlast, the reticular stimulation. Wheter the cat was inclined to doze during these short lasting episodes of EEG synchronization is simply a hypothesis, which should be tested with more refined behavioral technique.

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[1] See Hara, Favale, Rossi and Sacco (21 and 22) for what concerns the effects of low frequency reticular stimulation during the deepest stage of sleep with desynchronized EEG.

## DISCUSSION

The experiments reported here have been mainly concerned with the EEG response to low frequency stimulation of the reticular substance of the brain stem in intact unanesthetized free-moving cats. The chief result obtained is that generalized slow high amplitude waves similar to those spontaneously occurring during sleep are elicited by such stimulation. Moreover, some observations have been made suggesting that behavioral signs of sleep appear whenever the EEG synchronization has a tendency to outlast the reticular stimulation.

Previous data of other authors and of ourselves showing that EEG (48, 18, 14, 7, 31, 33, 43, 42, 17, 49, 34) and behavioral sleep (12, 29, 14, 45, 44) may follow adequate electrical stimulation of brain stem reticular structures have thus been confirmed. In addition, the following findings have been obtained: *a*) EEG synchronization is induced by low frequency stimulation of almost all the reticular sites explored, at midbrain, pontine and bulbar level; *b*) EEG synchronization is elicited only in particular experimental conditions, *i. e.*, when the animal is quiet and the spontaneous EEG rhytms are chiefly desynchronized with an intermingling of scattered burst of slow activity; *c*) induced EEG synchronization appears at first only during the period of reticular stimulation, but, with the repetition of the stimulation, it becomes evident also in the intervals between single stimulation periods and outlasts their end; *d*) behavioral changes suggestive of sleep are manifest only following repeated reticular stimulations, *i. e.*, when synchronized rhythms are almost continuously present in the EEG.

The present discussion will be centered on the three following main topics: 1) identification of the brain stem structures yielding EEG synchronization and sleep; 2) " phasic " and " tonic " phenomena of EEG synchronization and their relation to behavioral sleep; 3) mechanisms involved in the production of EEG and of behavioral signs of sleep by low rate reticular stimulation.

1. *Identification of the brain stem structures yielding EEG synchronization and sleep.* — It is well known that a number of fibres of extrareticular origin pass up and down through the reticular formation to connect spinal cord, brain stem and higher encephalic centers (see 46). Thus the hypothesis may be advanced that the

effects observed in our experiments were due to stimulation of these fibres and not of reticular neurones.

At least three possibilities must be taken into account: *a*) orthodromic excitation of fibres descending from higher centers, leading to spinal effects which might in turn influence the brain through central or peripheral loops; *b*) antidromic excitation of these same descending fibres; *c*) orthodromic excitation of sensory fibres ascending from spinal cord and/or brain stem sensory structures.

While the first of these possibilities is crucially disproved by the persistence of the EEG-synchronizing effect after high cervical transection, only indirect data are available to exclude the other two possibilities. These data may be summarized as follows. 1) Evoked potentials were never observed in the sensory-motor cortex following single shock stimulation of the reticular sites yielding EEG synchronization, while evoked cortical potentials are on the contrary always recorded following single shocks to the bulbar pyramis (which may also give rhythmic muscular contractions) or to the classical sensory paths. 2) Although a number of sensory fibres arising in the spinal cord or in the lower brain stem have been reported to ascend through the reticular formation, they are not so widely distributed as to cover the whole extent of it (see 46 for references), while EEG synchronization was elicited by stimulating the reticular formation in its medial and lateral aspects, both dorsally and ventrally, at mesencephalic, pontine and medullary levels. 3) EEG and behavioral effects quite different in type, to be described in a subsequent article, were recorded when low frequency stimulation was intentionally applied to sensory tracts or nuclei of the brain stem. On the whole, therefore, we think it likely that our results were due to stimulation of reticular neurones.

According to our experience, EEG synchronization and behavioral signs of sleep are obtainable by stimulating points of the reticular formation of the entire brain stem (Fig. 5). However, the question whether these effects may be elicited from the *whole* of the reticular substance cannot be answered since some reticular districts were not explored, as for instance the nuclei reticularis paramedianus and reticularis lateralis, both located in the medulla and projecting to the cerebellum. It may be worth noting that Magnes, Moruzzi and Pompeiano (35) succeeded in producing EEG synchronization by low frequency stimulation of the nucleus reticularis ventralis of the medulla (which gave positive results also in our experiments) but not from the adjacent nucleus reticularis paramedianus. Moreover, the responses obtained by stimulating at pontine and midbrain levels were not necessarily due to excitation of the local reticular neurones. They might have arisen from co-stimulation of ascending fibres arising from medullary reticular nuclei, an hypothesis that would actually fit the results of other experiments suggesting that the EEG-synchronizing structures of the brain stem, or at least the most important ones, are located in the caudal pons and in the medulla (8, 9, 15).

From an examination of our Fig. 5, which shows how widely distributed are the reticular sites from which positive effects were obtained, one might get the impression that the induced EEG and behavioral phenomena are dependent more on the modality of stimulation (as for instance low rate of pulse waves) than on the stimulated structural substrate. This would be, however, a false impression. Although the modality of electric stimulation is of the greatest importance, there is no doubt that the effects obtained from the reticular formation have never been produced by exciting, with the same parameters of electric stimulation, in the same animal and in the same experimental session, non-reticular structures of the brain stem. Furthermore, also within the boundaries of the reticular formation, some points have been found which did not give any EEG synchronization nor behavioral signs of sleep.

On the other hand, the results reported above do not imply that EEG synchronization and behavioral signs of sleep are obtainable *exclusively* from stimulation of the reticular substance. The recent work of Magnes, Moruzzi and Pompeiano (35), and personal data to be reported in a subsequent paper, clearly show that EEG synchronization may follow low frequency stimulation of other brain stem structures.

2. *" Phasic " and " tonic " phenomena of EEG synchronization and their relation to behavioral sleep.* — In their study of the habituation of EEG arousal produced by sensory stimuli, Sharpless and Jasper (47) made some interesting observations which led them to make a distinction between " phasic " activation of the EEG, characterized by short latency and never overlasting the sensory stimulus, and "tonic" activation, with longer latencies and persisting for several minutes after the end of the stimulation. The same distinction might be justified for the opposite effect, namely EEG synchronization elicited by trains of low rate electrical pulses applied

at regular intervals to the reticular formation. We have observed: *a*) bursts of EEG synchronization appearing at the beginning of each stimulating train and disappearing at the end of it, or a few seconds thereafter; *b*) a slow development of long lasting synchronization, which becomes evident after several stimulating trains have been applied and persists for several minutes after the end of stimulation. In analogy with the distinction made by Sharpless and Jasper for the EEG arousal reaction, these two effects may be qualified respectively " phasic " and " tonic ".

If this distinction between EEG-synchronizing effects is accepted and a correlation is made between EEG and behavioral responses to low frequency reticular stimulation, it soon becomes apparent that the only manifest behavioral sleep-like changes are concomitant with the " tonic " EEG reaction. As a matter of fact, other authors who reported behavioral signs of sleep following stimulation of the thalamus (29, 30, 37, 3, 4, 38, 44) and of brain stem structures (12, 29, 14, 45, 44) also pointed out that the animal assumed a typical sleep attitude only after repeated stimulations, and that, once induced, the sleep outlasted the stimulus. The hypothesis might be advanced that the " phasic " EEG synchronization is the electrophysiological correlate of short episodes of drowsiness occurring before the onset of sleep. However, clear cut behavioral changes were not observed in these experimental conditions. It is a common experience that weak sensory stimuli or threshold electrical excitation of the reticular activating system, when applied during sleep, *i. e.* on a background of EEG synchronization, may produce an EEG arousal which does not outlast the stimulus. Usually no behavioral signs of arousal can be seen in these conditions. Nonetheless, it would be hard to maintain that sleep is not modified during the short period of EEG activation. It is likely that, in physiological conditions, changes in the cerebral electrical activity always reflect changes in behavior. Therefore, a simple explanation of the apparent dissociation between EEG and behavioral effect of the reticular stimulation would be that our methods of recording behavior are much too rough, in relation to our EEG techniques, to detect the very first signs of either the onset of sleep or of arousal from sleep.

3. *Mechanisms involved in the production of EEG and behavioral effects of low rate reticular stimulation.* — At the end of the first part of the discussion, the assumption was made that the EEG

synchronization and the behavioral signs of sleep were primarily reticular effects, in the sense that they were not likely to be due to spread of current to neighboring structures or to co-stimulation of through fibres. We are confronted with two possibilities: *a*) the neurones of the reticular activating system were depressed by the repetitive application of trains of low rate pulses, either through transient damage produced by current flow or by synaptic fatigue; *b*) the neurones involved were not those of the activating system but different reticular units endowed with an antagonistic (*viz.*, EEG-synchronizing and sleep-inducing) influence on the cerebrum.

We think that the following facts could hardly be explained by a primary depression of the activating system: *a*) the strict temporal relation between stimulation and EEG response of the "phasic" type; *b*) the quick and complete reversibility of the "tonic" EEG synchronization and of the related manifestations of sleep; *c*) the fact that EEG synchronization and behavioral sleep can be produced by stimulating reticular sites lying in the most caudal part of the activating system. Concerning point *c*) it will be recalled that when the medulla and the lower pons are separated from the cerebrum (midpontine pretrigeminal cat), the upper part of the brain stem is more than adequate to maintain low voltage fast activity in the EEG and behavioral signs of wakefulness (8).

We may assume, therefore, that the EEG and behavioral results of our experiments were due to the *excitation* of reticular neurones having EEG-synchronizing and sleep-inducing function. Here again, two suggestions may be made to explain the possible ways in which this function is fulfilled (see also 40, 35): *a*) through an inhibitory action on the activating system leading to a "release" of EEG-synchronizing and sleep-inducing influences originating elsewhere, for instance in the thalamus; *b*) through a primary enhancement of those influences. In the discussion of their results of low frequency stimulation of the nucleus of the solitary tract and nucleus reticularis ventralis, Magnes, Moruzzi and Pompeiano (35) pointed out that the "release" hypothesis would hardly explain why slow high amplitude waves appeared so soon after the first stimulating pulses. We agree with this opinion and we think therefore that the ascending volleys elicited by the low rate stimulation of the brain stem might exert a facilitatory influence on the higher subcortical structures responsible for EEG synchronization, thus explaining the "phasic" EEG effects. On the other hand, the slow development and the long duration of the " tonic " EEG response and the gradually occurring changes in behavior related to it would perhaps find better explanation in the first hypothesis, *viz.*, that the activating system may be actively inhibited by the synchronizing reticular structures. Magnes, Moruzzi and Pompeiano (35) have pointed out that the two hypotheses are not mutually exclusive, since the volleys elicited by low rate reticular stimulation might drive the spindling mechanism and also, after repeated trains of pulses, inhibit the activating system, thereby releasing this same spindling mechanism. Actually, we think that the following considerations support the hypothesis that both mechanisms might have been responsible for the results of our experiments: *a*) " tonic " synchronization and behavioral sleep never develop without being preceeded by a lapse of time of variable duration in which only " phasic " EEG responses are evoked by low frequency reticular stimulation; *b*) the electrographic appearance of " phasic " and " tonic " EEG responses is quite the same.

On the other hand, the possibility that in some experimental conditions only one or the other of the two suggested mechanisms of EEG synchronization and sleep may be active should not be discarted. This would actually explain why in the studies of several authors dealing with EEG and/or behavioral responses to low frequency reticular stimulation, only " phasic " (7, 33, 43, 42, 34, 49) or only " tonic " (29, 12, 14, 38, 44) phenomena were observed.

## SUMMARY

EEG and behavioral effects induced by low rate stimulation of the brain stem reticular formation have been studied in unanesthetized free-moving cats carrying chronically implanted electrodes.

1) Trains of electrical pulses at a frequency of 4-12/sec produce: *a*) generalized bursts of high voltage slow waves, similar to those occurring during sleep and limited to the period of stimulation (" phasic " effect), and, after several trains, *b*) patterns of EEG synchronization and behavioral changes suggestive of sleep which outlast the stimulation (" tonic " effect).

2) Low rate of stimulation and a proper EEG and behavioral background (relaxed wakefulness) are necessary to obtain both effects.

3) Almost all reticular sites explored, in the midbrain, pons and medulla, give these effects.

4) EEG synchronization is still elicited after retrobulbar transection (*encéphalé isolé* preparation) and curarization. It disappears after electrolytic destruction of the tissue surrounding the stimulating electrode.

5) The EEG and behavioral effects are probably due to the excitation of reticular neurones having an EEG-synchronizing and sleep-inducing influence.

6) The possible mechanisms underlying the observed phenomena are discussed.

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# THE EFFECT OF CORTICAL AND HIPPOCAMPAL SPREADING DEPRESSION ON ACTIVITY OF BULBOPONTINE RETICULAR UNITS IN THE RAT

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## INTRODUCTION

The analysis of interrelations between various parts of the central nervous system has focussed the attention of neurophysiologists upon methods, aimed at overcoming the drawbacks of the classical extirpation techniques. The immediate results of a lesion are complicated by the general effects of surgery and subsequent compensatory processes may mask the original defect. There have been a number of attempts therefore to perform functional ablation of neural tissues instead of anatomical ablation in analysing complex neural processes. Reversible impairment of neural activity in circumscribed brain regions was achieved more or less successfully by local application of drugs (15), local cooling (10) and electrically evoked afterdischarges (12). The use of Leão's spreading depression for similar purpose was recently suggested (7, 4).

The spreading depression of Leão (13) is a characteristic response of the cerebral cortex, and also of some other neural structures (16), to mechanical, chemical or electrical stimuli. In the stimulated region a decrease of spontaneous ECoG amplitude develops, which spreads very slowly with a velocity of 2-5 mm/min across the cortex. The front of the spreading ECoG depression is accompanied by a slow potential variation, characterized by a negativity of 5-10 mV followed after 1-2 min by positivity of a lower value. Electrical, vascular, biochemical and behavioural manifestations of spreading depression were recently reviewed by Marshall (16), Bureš, Burešová and Křivánek (5), and Bureš and Burešová (4).

Since both the behavioural and electrophysiological findings indicate severe impairment of cortical functions during spreading depression, this condition may be considered as a model of reversible decortication. Nevertheless the possibility must be taken into account that some of the behavioural effects of spreading depression (e. g., impairment of conditioned reflexes) are due not only to elimination of the cortex, but also to secondary changes evoked in remote regions of the brain either by the active components of spreading depression or by release of subcortical structures from tonic cortical inhibition.

The purpose of the present paper is to analyse the influence of spreading depression on the activity of the reticular neurons.

## METHODS

In adult albino rats 3 trephine openings 5 mm in diameter were made under ether anesthesia over the cerebellum and over the occipital regions of both cerebral hemispheres. Two trephine openings 3 mm in diameter were made over the frontal poles of the hemispheres. After cannulation of the trachea the rats were immobilised with *d*-tubocurarine, fixed in a stereotaxic apparatus and maintained under artificial respiration.

Steel microelectrodes, electrolytically sharpened to tips of 2-6 μ diameter and insulated with acetone varnish, were introduced with a microdrive into the bulbopontine reticular formation, approximately at Krieg's planes 10 and 11.

The unit activity was amplified with a condenser-coupled push-pull preamplifier and recorded on a dual beam CRO. Only well isolated units, showing activity 3-5 times higher than the maximal peak to peak noise, were used for further analysis. The amplitude of response usually was greater than 250 μV. As a first step the effect of touch, pressure and proprioceptive stimuli was tested. Only those units which responded to stimulation of several skin areas and/or sensory modalities were considered as reticular. In approximately one third of the experiments the electrode position was verified by electrolytical microlesions and identified on Nissl stained serial sections.

Since spreading depression caused changes of spontaneous unit activity lasting often for half an hour, photographic recording of the responses throughout the whole experiment was impractical. The average firing rate was obtained, therefore, at one minute intervals with a decade electronic counter connected to the output of the CRO amplifier. In some cases the whole experiment was recorded with a tape-recorder (MOM-Budapest) connected in parallel to the counter. As distortion was negligible the spikes could be easily counted by replaying the tape.

Cortical spreading depression was evoked by application of 3 × 3 mm filter papers soaked with 1-2% KCl to the surface of the cortex exposed by the larger trephine openings. The slow potential wave of spreading depressions was led off through the small trephine openings with wick calomel-cell electrodes with physiological saline in the electrode vessel. A simple mechanical chopper changed the steady potential into rectangular pulses (5/sec), which were amplified with a conventional AC amplifier and recorded on the other beam of the CRO. The position of the spot was recorded every 30 or 60 sec on transparent paper.

In the experiments on hippocampal spreading depression, a stainless steel cannula (outer diameter 0.8 mm) was inserted under Pentothal anesthesia, with a stereotaxic machine, so as to reach a point corresponding to Krieg's coordinates AP + 4, L 4, V 4. The cannula was then fixed to the dry and roughened bone with acrylate cement. Two or three days later activity of reticular units was registered in the usual way. Hippocampal spreading depression was evoked by inserting a small KCl crystal through the implanted cannula into the hippocampus. After the end of the experiment the rat was killed by perfusion of the carotids with formaline and the location of the cannula was verified anatomically.

In some cases both hippocampal and cortical spreading depression were evoked in turn, in order to compare their effects on the activity of the same unit.

## RESULTS

1. *The reticular effects of cortical spreading depression.* — In 34 rats the activity of 119 reticular units was examined during cortical spreading depression. Spontaneous activity was first recorded, for at least 15 minutes. Cortical spreading depression was evoked in one hemisphere only when the reticular activity had become sufficiently stabilized.

In order to systematize the rather heterogeneous results obtained, the reactions were classified according to the following criteria.

i) Immediately before the local application of the KCl solution, average firing rate and standard deviation were computed for 10 periods, each lasting one minute. The unit was considered as responding to spreading depression when the firing rate in any one minute period of the two minutes before or after the cortical slow potential wave had reached its maximum differed by more than 3 standard deviations from the control mean frequency. In some cases there was an increase in frequency, in others a decrease.

ii) The reaction was considered as short-lasting when the increased or decreased firing exceeded the three standard deviations range for four minutes or less. Reactions lasting more than four minutes were classified as long-lasting.

The activity of approximately 80% of the units was changed during cortical spreading depression. Typical reactions obtained in three different experiments are illustrated by Figs. 1-3. The most common reaction was a short-lasting increase of activity shown in

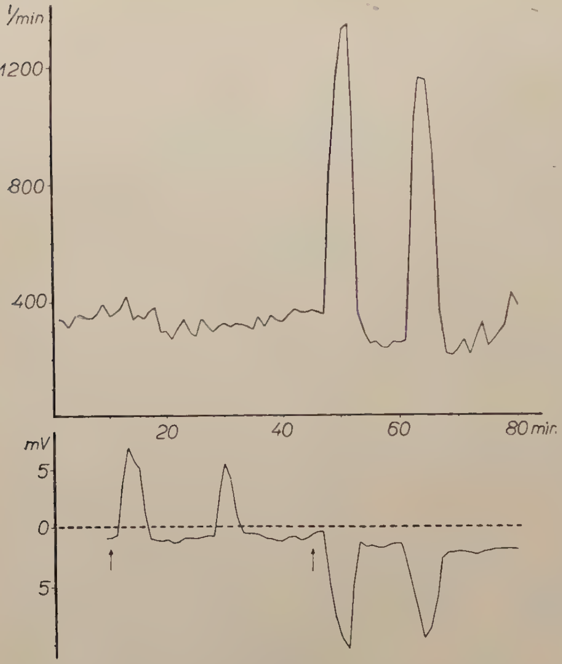

Fig. 1. – *Short lasting increase of activity evoked in a bulbar reticular unit by cortical spreading depression.*

Curarized rat. Above: firing rate (spikes/min). Below: steady potential changes registered in frontoparietal cortical regions. Negativity of the right hemisphere downwards.

Application of 1% KCl to the homolateral (left) hemisphere (1st arrow) did not change the unit activity, while spreading depression evoked in the contralateral (right) hemisphere (2nd arrow) was accompanied by increased firing.

Fig. 1. Fig. 2 represents a long-lasting increase reaction and Fig. 3 a long-lasting decrease reaction.

Sometimes a unit responded to both homo- and contralateral

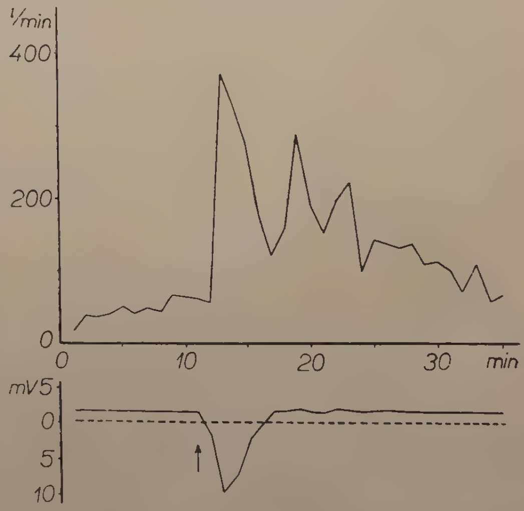

Fig. 2. – *Long lasting increase of activity evoked in a pontine reticular unit by cortical spreading depression.*

Curarized rat. Above: firing rate (spikes/min). Below: steady potential changes registered in frontoparietal cortical regions. Negativity of the right hemisphere downwards. Arrow indicates application of 1% KCl to the contralateral cortex.

spreading depression with the same type of reaction. In other experiments the unit responded only to spreading depression evoked in one hemisphere (contralateral or homolateral to the reticular microelectrode) and remained unaffected by spreading depression evoked in the other hemisphere (e. g., Fig. 1). There was no systematic difference between the types of unit reactions to homo- or contralateral spreading depression.

Fig. 4 shows the location of same anatomically verified sites of recording and the responses obtained from these points to homo- or contralateral spreading depression.

2. *The reticular effect of hippocampal spreading depression.* — In contradistinction to the effects of cortical spreading depression, most of the reticular units (approximately 75%) were unaffected by spreading depression in the hippocampus. Usually a spreading depression wave starts 1-2 minutes after insertion of the KCl crystal into the hippocampus and further spreading depression waves appear at approximately 5-10 minutes intervals (6, 23). When the firing rate remained within the three standard deviations range for 5 min after KCl application, the unit was considered as unresponsive.

## Discussion

Corticoreticular connections have been demonstrated repeatedly both by anatomical and electrophysiological techniques (see 21). In the cat most of the corticoreticular fibres come from the frontal cortex and terminate in the reticular formation of the pons and medulla. It seems that they are either collaterals of the corticospinal fibres or that they course along with the pyramidal pathway. According to Rossi and Brodal (20) fibres originating in other cortical regions also contribute to the corticoreticular projections.

The electrophysiological evidence (3, 2, 19, 22) is in agreement with the anatomical data, although the percentage of reticular units, which may be activated by cortical stimulation, depends considerably upon experimental conditions.

The relative number of units (80%) affected by cortical spreading depression in our experiments is high. Of course long lasting changes of spontaneous unit activity found in the present experiments can scarcely be compared with the short lasting effects due

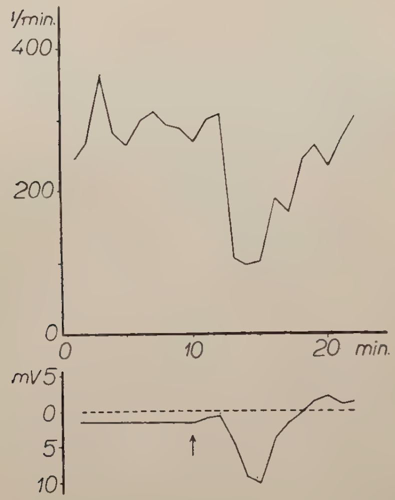

Fig. 3. – *Long lasting decrease of unit activity evoked in a bulbar reticular unit by cortical spreading depression.*

Curarized rat. Above: firing rate (spikes/min). Below: steady potential changes registered in frontoparietal cortical regions. Negativity of the right hemisphere downwards. Arrow indicates application of 1 % KCl to the homolateral cortex.

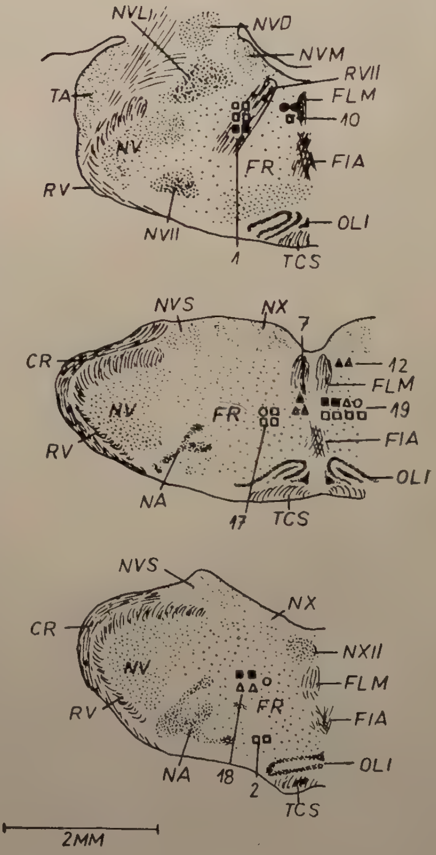

Fig. 4. – *Location of some anatomically verified sites of recording.*

Squares: increase reaction; triangles: decrease reaction; circles: no reaction. Empty symbols represent reaction to contralateral, black symbols to homolateral spreading depression. The drawings taken from serial sections correspond approximately to Krieg's frontal planes 9, 11 and 12. Magnification 15 X.

*Abbreviations used*: CR: corpus restiforme; FR: formatio reticularis; FIA: fibrae arcuatae internae; FLM: fasciculus longitudinalis medialis; NV: nucleus nervi trigemini; NVII: nucleus nervi facialis; NX: nucleus nervi vagi; NXII: nucleus ambiguus; NVD: nucleus vestibularis dorsalis; NVM: nucleus vestibularis medialis; NVL: nucleus vestibularis lateralis; NVS: nucleus vestibulairs spinalis; OLI: oliva inferior; RV: radix nervi trigemini; RVII: radix nervi facialis; TCS: tractus corticospinalis.

to strychnine application or electrical stimulation. Spreading depression is probably more effective than other forms of stimulation because it eventually invades all cortical areas. Especially in the small brain of a rat there is a period when practically the whole cortical surface is involved in spreading depression.

According to Baumgarten *et al.* (3) cortical inhibition of reticular units is much less frequent than cortical facilitation. The inhibition-facilitation ratio was 1:3.6 in our population of reticular units and 1:3 in the experiments of Scheibel *et al.* (22). Inhibitory reactions evoked by cortical stimulation are probably mediated by corticoreticular fibres forming inhibitory synapses with the reticular neurons. On the other hand, the decrease of spontaneous activity caused by spreading depression may be simply explained by reduction of the excitatory corticoreticular influences.

According to Rossi and Brodal (20) corticoreticular fibres originating in one hemisphere are equally distributed to both halves of the reticular formation. Baumgarten *et al.* (3) have found the reticular responses to cortical stimulation to be clearly bilateral. In our experiments too the same number of responding units was found homo- and contralaterally.

The effect of spreading depression on reticular units may be caused by elimination of the cortical inhibitory tonus demonstrated by Hugelin and Bonvallet (10). An increase of the spontaneous activity of reticular units would then constitute a release phenomenon. Since spontaneous ECoG and cortical unit activity are altered for 10-20 minutes by a single spreading depression wave, the frequent occurrence of short lasting reactions in the responding reticular units is at variance with the above explanation.

The alternative explanation is that the reticular reactions are due to cortical hyperactivity. Grafstein (8) has shown that discharge of unit activity lasting 2-3 sec accompanies the rising phase of the slow potential change of spreading depression in the cortex. Some 3-4 minutes are necessary for spreading depression to pass across the whole rat cortex. Since the corticoreticular connections show significant convergence, a single reticular unit may be bombarded during this period by impulses originating in different cortical areas. Such a mechanism may easily account for the short lasting reactions, the duration of which corresponds well with the passage of the slow potential wave across the cortex, but it cannot explain the long lasting ones.

In addition to the hypothesis of a neural mechanism to explain the influence of spreading depression on reticular units, nonneural mechanisms (e. g., circulatory changes in the brain stem) must also be taken into account. Further experiments are necessary to decide between these two possibilities, although obviously they are not mutually exclusive.

According to Adey *et al.* (1) the hippocampus can mediate at least some of the corticoreticular influences and direct or indirect hippocampal projections to the brain stem have been demonstrated by anatomical methods (17). Electrographic arousal caused by stimulation of the hippocampus was reported by Kaada (11) whereas Lissak *et al.* (14) failed to evoke arousal by hippocampal stimulation. Hernández-Péon and Haggbarth (9) found that brain stem activity was suppressed by stimulation of the hippocampus. These controversial results are probably due to varying effects produced by hippocampal stimulation, which may cause seizures spreding to distant areas.

Spreading depression in the hippocampus has the same features as spreading depression in the neocortex: suppression of spontaneous and evoked activity, occasional appearance of paroxysmal activity and slow potential changes (6, 23). Spreading depression evoked in the hippocampus does not spread to the cortex and vice versa. The fact that cortical and hippocampal spreading depression influence the activity of bulbo-pontine reticular units in different ways can not be explained, therefore, by different properties of spreading depression in these structures but rather anatomical and functional differences between corticoreticular and hippocamporeticular projections must be involved. The statement of Nauta (17) seems to be relevant, i. e., that none of the major limbic pathways extends beyond the level of the isthmus in the cat. In the rat (18) fibres originating in the septum reach the paramedian area of the pontine tegmentum. The lack of any functional influence of the hippocampus on the reticular units could then be simply ascribed to the absence of direct anatomical connections.

## SUMMARY

In unanesthetized curarized rats the response of bulbopontine reticular units to cortical or hippocampal spreading depression was studied.

Out of 119 waves of cortical spreading depression produced, 97 were accompanied by changes in reticular activity, corresponding closely to the slow potential waves in the cortex. Short lasting (4 minutes or less) increase in frequency of firing was the most common reaction, but short lasting decrease and long lasting (longer than 4 minutes) increase or decrease reactions were also observed. The reactions to homo- or contralateral spreading depression were similar.

Unlike cortical spreading depression, hippocampal spreading depression did not affect the activity of reticular units in most cases.

Changes of reticular activity evoked by cortical spreading depression may be due either to sudden elimination of tonic cortico-reticular influences or to the short lasting discharge of cortical units (2-3 sec) accompanying the front of the spreading depression wave.

## Acknowledgement

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# SYNCHRONIZATION OF THE EEG PRODUCED BY LOW-FREQUENCY ELECTRICAL STIMULATION OF THE REGION OF THE SOLITARY TRACT [1]

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## INTRODUCTION

A decrease in the tonic activity of the ascending reticular system is generally regarded as the main cause of sleep. The origin of the reduction of the ascending flow of reticular impulses is however unknown.

According to Bremer (8) a "process of deactivation *en avalanche*" might arise within the reticular system itself as a consequence of an intrinsic process of neuronal fatigue, possibly favoured by the absence of light and noise and by the decrease of the exteroceptive and proprioceptive barrage that occurs when an animal falls asleep. This hypothesis, however, does not necessarily conflict with the assumption that the activating reticular system might also be directly inhibited, or antagonized, by EEG synchronizing and possibly sleep inducing structures. Behavioral observations and EEG records suggesting the existence of these structures within the thalamus and the nucleus caudatus have been repeatedly described (see 28, 30). The present work is concerned only with the prob-

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[2] U. S. Public Health Service Special Fellow, on sabbatical leave from the Hebrew University, Jerusalem, Israel.

lem of the existence of EEG synchronizing structures in the brain stem, particularly in the medulla.

Our experiments were undertaken in the hope that proof of a more direct nature for the presence of these structures could be adduced by recording the EEG during direct electrical stimulation of the lower brain stem, in the region of the nucleus of the solitary tract. The reason for selecting this particular region was that this area receives afferent fibers from the baroceptors of the carotid sinus and arch of the aorta. It will be recalled that Koch (31) showed that distension of the carotid sinus may cause a sleep-like state in the unanesthetized dog or monkey, and Bonvallet, Dell and Hiebel (7) were able to produce EEG synchronization in the *encéphale isolé,* hence in complete absence of changes of blood pressure and respiration, by stimulating the baroceptors of the carotid sinus in the same fashion. There are a number of other studies pointing to inhibitory effects of baroceptor stimulation on the cerebral cortex and the somatic nervous system. Reference to these may be found in the recent monograph by Heymans and Neil (22). From the results of the experiments to be reported it will be seen that *selection of the region of the nucleus of the solitary tract was justified because its electrical stimulation at low frequency produced EEG synchronization.* However we do not mean to imply that the induction of sleep is solely a function of baroceptor activity, because the region of the nucleus solitarius is not under the influence of the baroceptive system alone; moreover, although our studies were limited to a very restricted portion of the brain stem, it will be seen that EEG synchronization was also obtained by stimulating medullary sites other than the sensory nuclei of the glosso-pharyngeal nerves.

Before passing on to a description of the experiments it may be well to mention some of the previous lines of evidence indicating the presence of EEG synchronizing structures in the lower brain stem.

*Behavioral signs of sleep following electrical stimulation of the brain stem* were observed in the cat by Hess (21) and Parmeggiani (42), and in the monkey by Proctor, Knighton and Churchill (43). These authors applied low rate pulses to the midbrain, in the unanesthetized, intact animal. Experiments by Bürgi and Monnier (11) showed, moreover, that cataleptic symptoms with somnolence occurred in the cat following electrical stimulation of the pontine reticular formation.

*EEG synchronization following electrical stimulation of the brain stem* has been reported by several authors. French, von Amerongen and Magoun (17) occasionally noted EEG synchronization following either high or low rate stimulation of the reticular formation. High rate stimulation of well delimited regions of the reticular formation in the cat was found by Ingvar and Söderberg (26) to be followed by flattening of the EEG and occasionally by the appearance of spindles, mainly after the end of the stimulation. Araki, Sakata and Matsunaga (3), Nakamura (39), O'Leary, Kerr and Goldring (40), Kuroki (33), Umbach (46) and Lynes (34) recorded widespread EEG synchronization during low rate stimulation of the midbrain. Because of their analogy with the classical effects obtained from the midline nuclei of the thalamus by Morison and Dempsey (38) these authors defined as recruiting or recruiting-like the EEG waves elicited by mesencephalic stimulation. However the typical recruiting increase of the amplitude of the synchronous waves was absent, or at least not clearly present, in the experiments of Kuroki (33), Nakamura (39) and Umbach (46).

*A correlation between behavioral manifestations of sleep and EEG synchronization* was reported in the experiments of Caspers and Winkel (13) and of Favale, Loeb, Rossi and Sacco (15, 16) which were performed on unanesthetized, free moving animals, rats and cats respectively. The first group of authors stimulated the midbrain tegmentum, while the second group stated that EEG synchronization could be obtained from widespread areas of the entire reticular formation.

*A tonic EEG synchronizing influence arising in the lower brain stem* was suggested by the observations of Batini, Moruzzi, Palestini, Rossi and Zanchetti (5) on the midpontine pretrigeminal cat. In contrast to the *encéphale isolé*, this preparation is in the waking state both behaviorally and with regard to the EEG for most of the survival time. Several controls (4, 6) indicate that structures lying between the level of the midpontine section and the caudalmost part of the medulla have a synchronizing influence on the EEG. Cordeau and Mancia (14) reached the same conclusion in a study of the EEG asymmetries appearing in cats which had undergone unilateral midpontine transections. Further experiments by Magni, Moruzzi, Rossi and Zanchetti (36) showed that small doses of thiopental, injected into the arterial circulation of the lower brain stem, induced EEG arousal. In this case it was suggested that tonically active EEG synchronizing structures in the caudal part of the brain stem were blocked by the drug. On the other hand intracarotid injections of small doses of the same substance produced EEG synchronization, and if the dose was small enough the spindling was restricted to the side of injection only.

## METHODS

*Encéphale isolé* cats were prepared under ether anesthesia, the spinal cord being sectioned at $C_1$ or $C_2$. Every effort was made throughout the experiment to maintain artificial ventilation and body temperature at their proper levels. Periodic controls of femoral arterial pressure were also made.

In order to reduce to a minimum noxious stimuli, likely to activate the animal, local anesthetics were routinely used on the wounds and at the places of application of the ear bars of the Horsley-Clark apparatus. Sometimes, however, despite these precautions, the EEG was found to be activated. Under these conditions one or more intravenous injections of thiopental-Na [1] in very small doses (0.5 - 2.0 mg per Kg body weight) produced a more synchronized background of electrocortical activity. In our last group of experiments the head was held in a Czermak holder, which probably causes less nociceptive effects than the Horsley-Clarke machine. It turned out that the region of the nucleus solitarius could be reached easily and precisely even without a stereotaxic procedure, by using the obex as a landmark, following suction of the posterior vermis to provide better exposure. In confirmation of Wang, Lin and Ho (50) and of Ho, Wang, Lin and Cheng (23) we found that the *encéphale isolé* cat often (thought not constantly) has a marked tendency towards drowsiness or sleep when nociceptive stimuli are carefully avoided. EEG arousal was actually routinely used in our experiments, as a reliable test of the good condition of the *encéphale isolé*.

Electrical stimulation was carried out with a Tektronix stimulator using square pulses at a frequency of 1-30/sec, 2-4 V in amplitude and 1-5 msec in duration, applied through concentric needle electrodes (30.000-50.000 $\Omega$ resistance). The EEG was recorded on an ink-writing oscillograph from screw electrodes inserted through boreholes in the skull over the frontal, parietal, temporal and occipital areas. Electrocardiogram was recorded in most of the experiments. When desynchronization of the EEG was required, acoustic stimulation (short blast on whistle), trigeminal stimulation (rubbing the nose) or electrical stimulation of the reticular formation with 30-300 pulses per sec was employed.

In a number of experiments, acute or chronic electrolytic lesions of the brain stem were performed in an attempt to identify the pathways responsible for the EEG synchronizing effects observed upon stimulation of the region of the nucleus solitarius.

At the end of each experiment the stimulated points were destroyed electrolytically and the histological controls were made upon alternate Nissl and Weil stained sections.

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[1] Kindly supplied by Farmitalia, Milan.

## RESULTS

1. *Types of response elicited by stimulating the region of the nucleus of the solitary tract and the cortical areas affected.* — Detailed descriptions of the various types of response elicited will be presented in the forthcoming sections. Generally speaking, however, the synchronizing responses elicited by low frequency stimulation of the region of the nucleus of the solitary tract were large amplitude, rounded waves of long duration occurring at the same frequency exhibited by the brain when spontaneous spindling was present. In fact it was usually impossible to distinguish the pattern elicited by electrical stimulation of these medullary structures from the spontaneous spindle bursts that occasionally appeared in the same cat. There was little doubt, however, that the episodes of EEG synchronization were causally related with the trains of electrical pulses applied to the medulla, since the response was coincident with the stimulation and could be obtained several times, and in a thoroughly predictable manner. This type of synchronization differs from that obtained by stimulating the midline nuclei of the thalamus: i) because there was no evidence of recruitment of cortical neurons even with threshold stimuli; ii) because the repetition rate of the elicited slow waves was unrelated to that of the stimulus over a fairly wide range; and finally, iii) because in many experiments intermittent synchronization of the EEG persisted for some time after interrupting the electrical stimulus, though prior to stimulation the background of activity had been desynchronized. The same points which yielded synchronization when stimulated at low rates, usually produced arousal when stimulated at higher frequencies. On the other hand certain points were found in very close proximity which as the result of low frequency stimulation elicited arousal (see section 5).

The synchronization elicited was always bilateral and was usually generalized to the entire dorsal aspect of the neocortex. In some cases it was weak or absent in the frontal records but it was always marked in the occipital and temporo-parietal leads. The monopolar records of Fig. 1 *B* are a typical example of the responses obtained and of their generalization. The similarity between the elicited response and the inherent spindling is also evident particu-

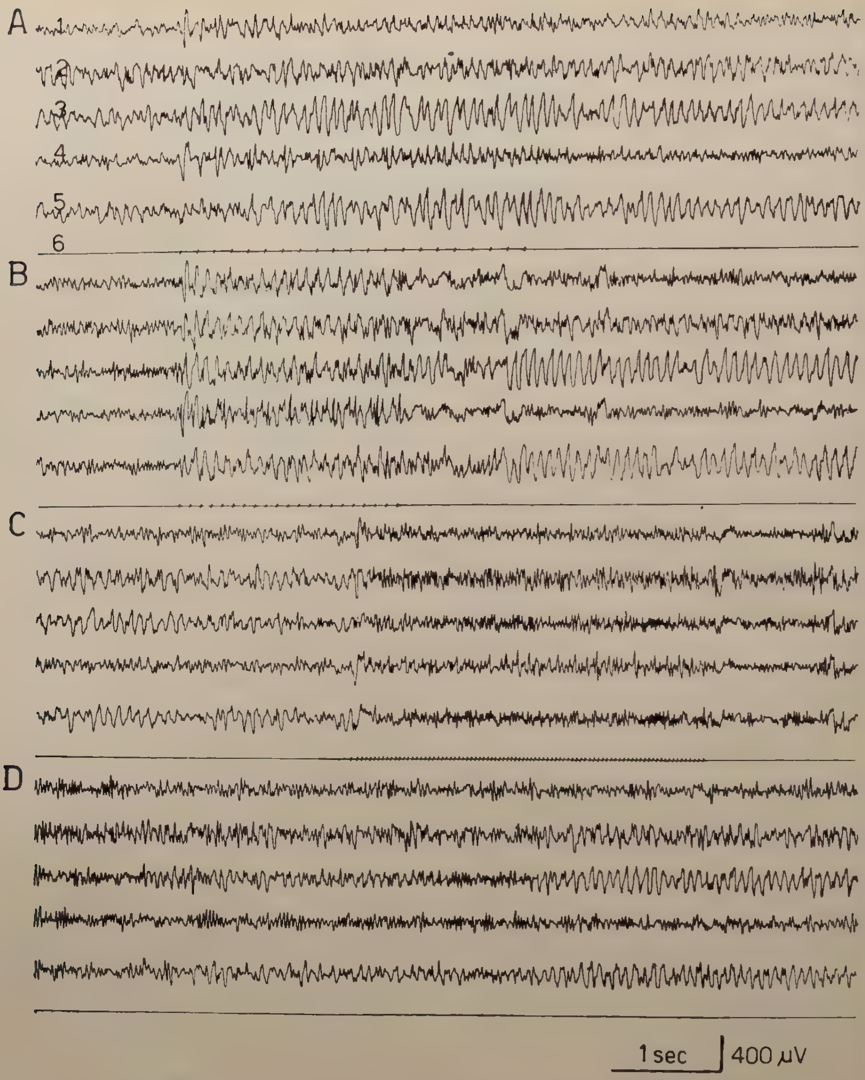


Fig. 1. – *Generalized synchronization of EEG elicited by stimulation of nucleus of solitary tract.*

*Encéphale isolé* cat. Stimulation with rectangular pulses of 4 V, 5 msec. *Monopolar records* (negativity upwards). 1: right parietal; 2: right temporal; 3: right occipital; 4: left parietal; 5: left occipital; 6: stimulus marker. *A*: stimulation at 6/sec; EEG rate in occipital leads 11-12/sec during and after stimulation. *B*: stimulation at 10/sec; EEG rate 10/sec during stimulation, 12/sec after stimulation. *C*: arousal during stimulation at 26/sec. Spontaneous synchronization with EEG rate 12/sec occurs in *D*. *C* and *D* are continuous records. Note that the spontaneous synchronization (*D*) and the after-effect of the stimulation (*A* and *B*) are particularly evident in the occipital leads.

larly in the occipital leads. The records of Fig. 2 were taken from the same cat and illustrate the precise localization of the points stimulated. This will be returned to in chapter 6.

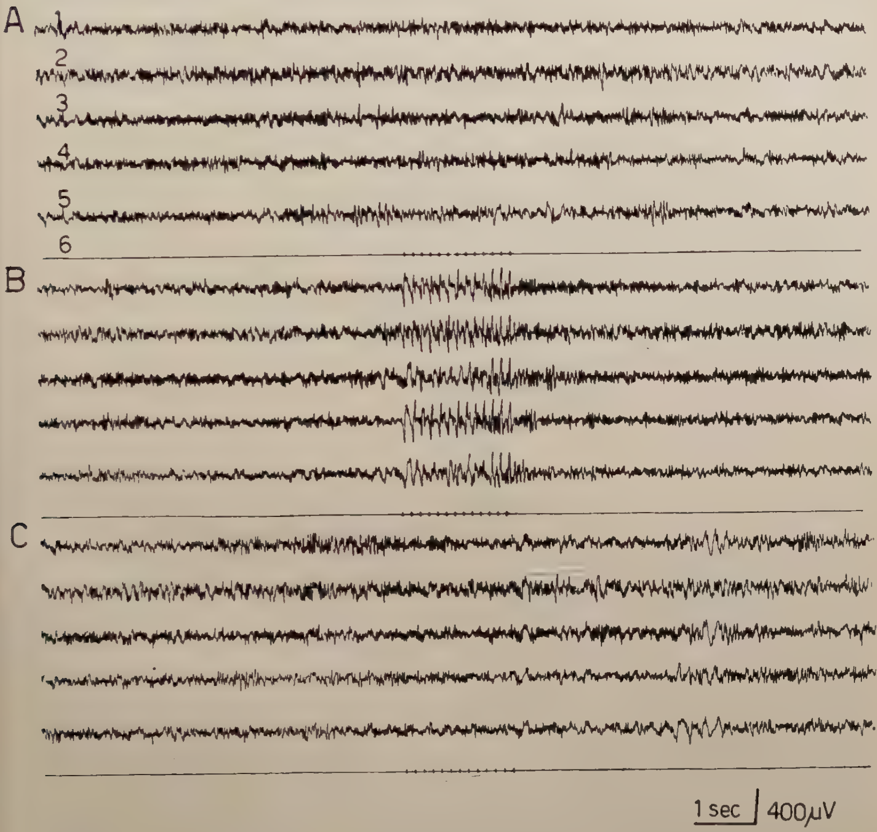


Fig. 2. – *Delimitation of point eliciting EEG synchronization.*

Same cat as Fig. 1. (Voltage and pulse duration same; pulse rate: 7/sec). *A*: stimulation at point 1 mm dorsal to that in *B* does not cause synchronization. *B*: stimulation of nucleus of solitary tract elicits synchronization (this is the same point that was stimulated to produce the records of Fig. 1). *C*: stimulation at point 1 mm ventral to that in *B* fails to produce synchronization.

2. *Influence of EEG "background" on synchronizing responses.* — It is well known that the *encéphale isolé* cat alternates between periods of sleep and wakefulness, but it should be pointed out immediately that in order to elicit EEG synchronization, by electrical stimulation of the medullary structures, a certain type of "back-

ground" is necessary. Using the pattern of spontaneous electrocortical activity as an index of this "background", it was found that when the degree of activation of the EEG was excessive, the prospects of achieving synchronization were very small indeed. On the other hand a strongly synchronized EEG was indicative of a state in which further synchronization could not be obtained. High voltage slow waves were most easily reproduced when the EEG record showed just a faint suggestion of spontaneous synchronization, though in many cases the response was obtained on a background of arousal. As indicated in the section on Methods it is of the greatest importance to provide the proper environment for the cat in order to achieve synchronization. Thus pain, excessively low or high body temperatures, high $CO_2$ and low $O_2$ tensions as well as visual, acoustic and mechanical stimuli from the environment should be avoided.

Some examples of the importance of the EEG "background" follow. Fig. 3 (record *A*) is an example of EEG synchronization elicited by stimulation of the region of the nucleus of the solitary tract at 10/sec. It will be noted that synchronization outlasts the period of stimulation. Between record *A* and *B* the cat was aroused by an acoustic stimulus, so that stimulation of the same point, with the same parameters of stimulation, 30 seconds after the acoustic stimulus, failed to produce synchronization (record *B*). In record *C*, which is a direct continuation of *B*, a spindle or two are evident at the beginning of the record indicating that the cat is returning to the "drowsy" state which was present prior to the acoustic stimulus. At this point electrical stimulation of the medullary structures again readily produced synchronization of the EEG as shown in *C* and in *D*. A similar example is illustrated in Fig. 8, record *A*, *B* and *C*. Fig. 8 *D* shows how the synchronizing influence can be disrupted by a strong acoustic stimulus, though in this case synchronization reappeared immediately once the acoustic stimulus was terminated. This may be attributed to the overwhelming effect of the synchronizing process due to the continuous electrical stimulation of the medullary structures, before, during and after the acoustic arousal.

Despite all the above mentioned precautions to provide proper conditions for producing synchronization, in some experiments the EEG remained fully activated. So as not to lose these experiments a small dose of thiopental-Na (0.5 - 2.0 mg/Kg body weight) was

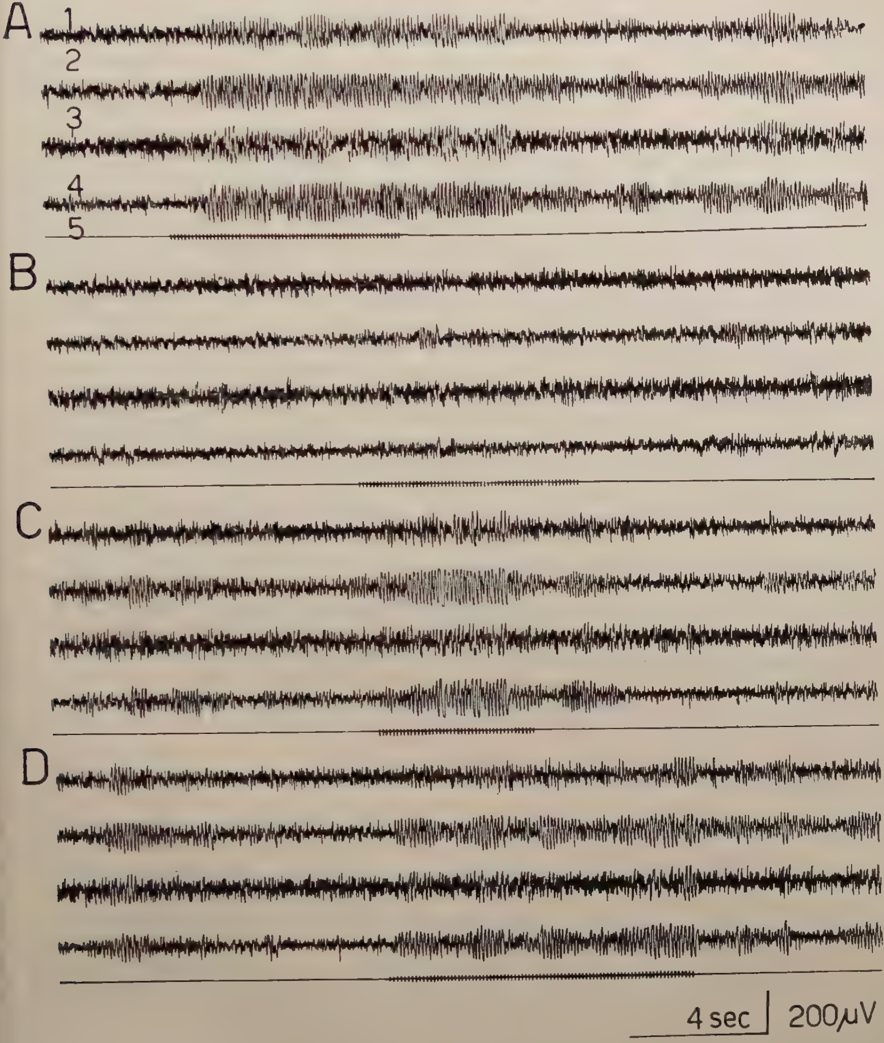


Fig. 3. – *Synchronization of EEG elicited by low rate stimulation of region of nucleus of solitary tract.*

*Encéphale isolé* cat. Stimulation with rectangular pulses of 3 V, 5 msec. *Bipolar records*: 1: right parieto-temporal; 2: right temporo-occipital; 3: left parieto-temporal; 4: left temporo-occipital; 5: stimulus marker. *A*: stimulation at 10/sec: EEG synchronization outlasting stimulus. *B*: same stimulation at 10/sec, 30 sec after acoustic stimulus: no effect. *C*: continuation of *B*; same stimulation at 10/sec: response with shorter aftereffect. *D*: continuation of *C*; same stimulation at 10/sec yields response comparable to that recorded in *A*. Note waxing and waning of the response.

injected into the femoral vein. This procedure usually provided a suitable EEG background so that synchronization could be obtained by electrical stimulation of the medullary structures. It is important to point out that the amount of thiopental administered should

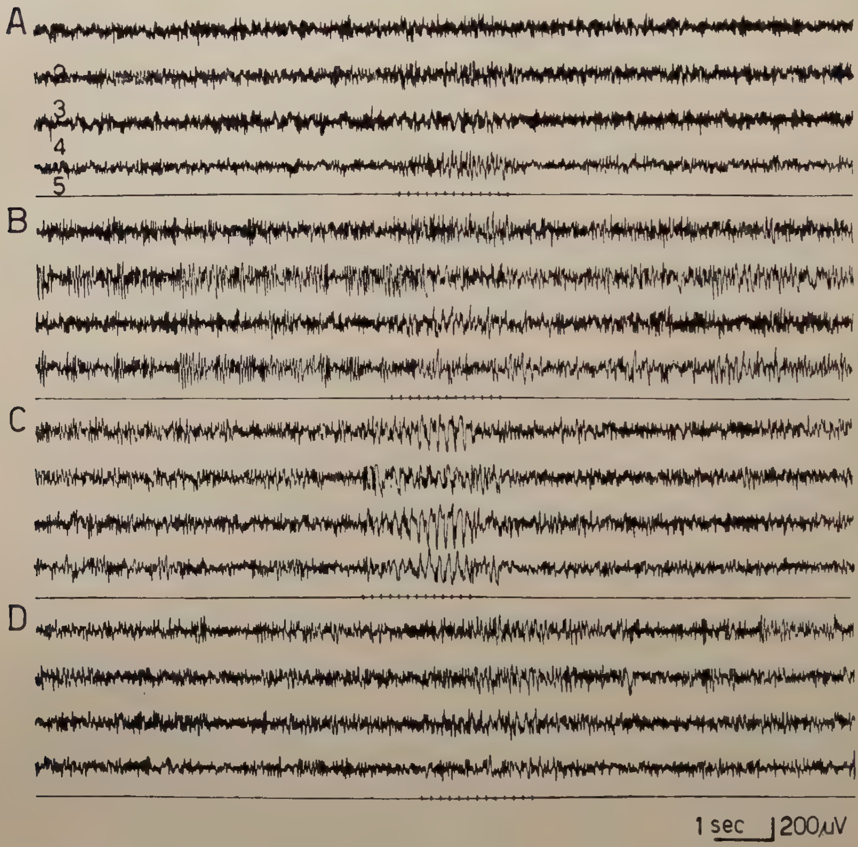


Fig. 4. – *Effect of intravenous pentothal on synchronization elicited by stimulation of region of solitary tract.*

*Encéphale isolé* cat. Stimulation with rectangular pulses of 3 V, 3 msec, 6/sec. *Bipolar records.* 1: right parieto-temporal; 2: right temporo-occipital; 3: left parieto-temporal; 4: left temporo-occipital; 5: stimulus marker. *A*: before pentothal. *B*: 20 sec after injection of 0.5 mg pentothal/kg into femoral vein. *C*: 80 sec after *B*. *D*: 40 sec after C.

not be so great as to produce frank spindling of the EEG, for then excessive synchronization results, and it obviously becomes impossible to achieve further effects by electrical stimulation of the medullary structures (Fig. 4 *B*). The optimal conditions for eliciting EEG synchronization with low rate electrical stimulation may then appear fleetingly during the falling phase of anesthesia (Fig. 4 *C*). Though thiopental is classified as one of the ultra-short acting barbiturates it is interesting to point out that in a few cats, which were remarkably resistant to synchronization, after 2 or 3 injections of thiopental the EEG began to show a " drowsy " pattern.

3. *Relationship between rate of medullary stimulation and the rhythm of the synchronizing response.* — In the majority of our experiments the frequency of the EEG during spontaneous episodes of synchronization lay between 10-12/sec, although in a few cats this rhythm was either faster or slower. In a series of experiments, in which the medullary structures were stimulated at increasing stimulation frequencies, it was found that so long as the stimulation rate did not exceed the spontaneous spindling rate, the frequency of the induced synchronization corresponded to the inherent EEG frequency. Stimulation at slightly higher frequencies, however, caused the repetition rate of the response to follow that of the stimulus, and further increases in stimulation frequency produced EEG arousal. The records of Figs. 5 and 6 taken at short time intervals from the same cat as those in Figs. 3, 6, 7 and 8 illustrate these points clearly.

Figs. 5 *B-C*, 6 *A-E* show the results of varying the stimulation frequency between 1 and 31/sec. In this particular cat the inherent frequency of the synchronous waves lay between 10 and 12 per second. When the electrical pulses were applied at the rate of only 1/sec, each stimulus was followed by a burst of rhythmic waves. This may be similar to the well known phenomenon of tripping of the spindle bursts, frequently reported in the experiments of stimulating the intralaminar nuclei of the thalamus (38, 27, 28). Repetitive stimulation at a frequency of 6 and 11/sec (Figs. 5 *C*, 6 *A*) produced bursts of synchronous waves which did not greatly outlast the end of the stimulation. This phenomenon is different from a recruiting response because i) the synchronization was already full sized after the first shock and ii) the induced rhythm remained that of the spontaneous rhythms or of the tripped spindles, and actually was about the same when the stimulation rate was 6 and 11/sec. However when the frequency of stimulation was raised to 18/sec the inherent rate of the brain rhythm was lost, and a 1:1 ratio between the stimulus rate and that of the response ensued;

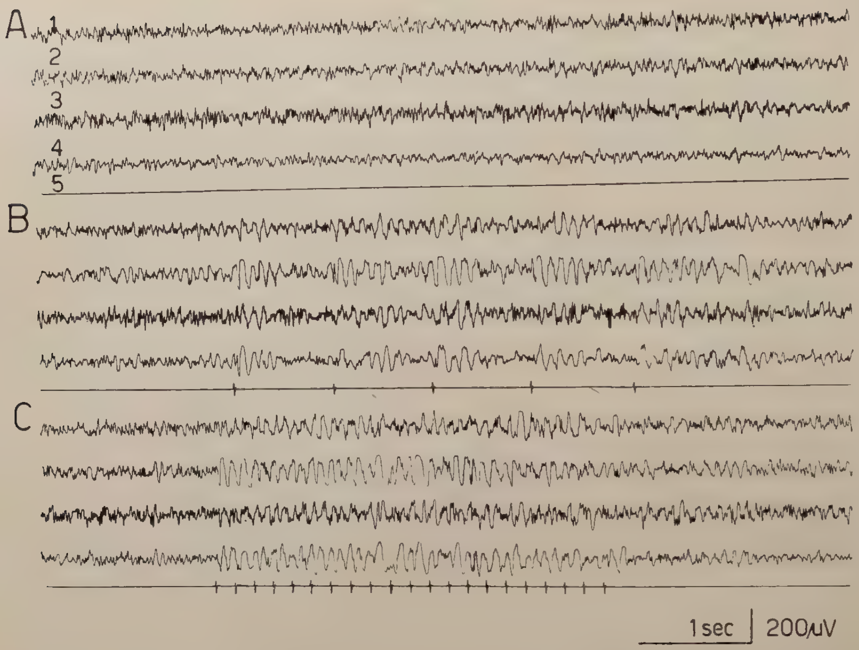


Fig. 5. – *Effect of changing stimulation rate on synchronization of EEG.*

Same cat as Fig. 3 (voltage and pulse duration same). *A*: spontaneous activity. B: spindle tripping elicited by stimulation at 1/sec.C: stimulation at 6/sec yields synchronous waves at the rate of 11/sec. Note that this rate is the same as that of the tripped spindles (*B*).

furthermore the response did not persist as long as the stimulus. At 20/sec (Fig. 6 *D*) synchronization was absent and there may even be a suggestion of arousal. Stimulation at 31/sec (Fig. 6 *E*) gave clear-cut EEG desynchronization. Results such as those reported in Fig. 5 are observed only when the animal has a marked tendency to EEG synchronization. When the tendency is less developed, the stimulation at 1/sec may be ineffective, and yet the optimum rate

Fig. 6. – *Effects of stimulation of region of solitary tract at higher rates.*

Same cat as Fig. 3 (voltage and pulse duration same). *A*: marked synchronization at 11/sec elicited in temporo-occipital leads by stimulation at 11/sec. *B* and *C*: stimulation at 18/sec yields short lasting synchronization of lower voltage and higher frequency (18/sec) in the same leads (*B*); the inherent rhythm at 10/sec appears after stimulation (*C*). *D*: electrical pulses at 20/sec have no synchronizing effect during stimulation, but after-effect is present as in *C*. *E*: arousal produced by stimulating at 31/sec.

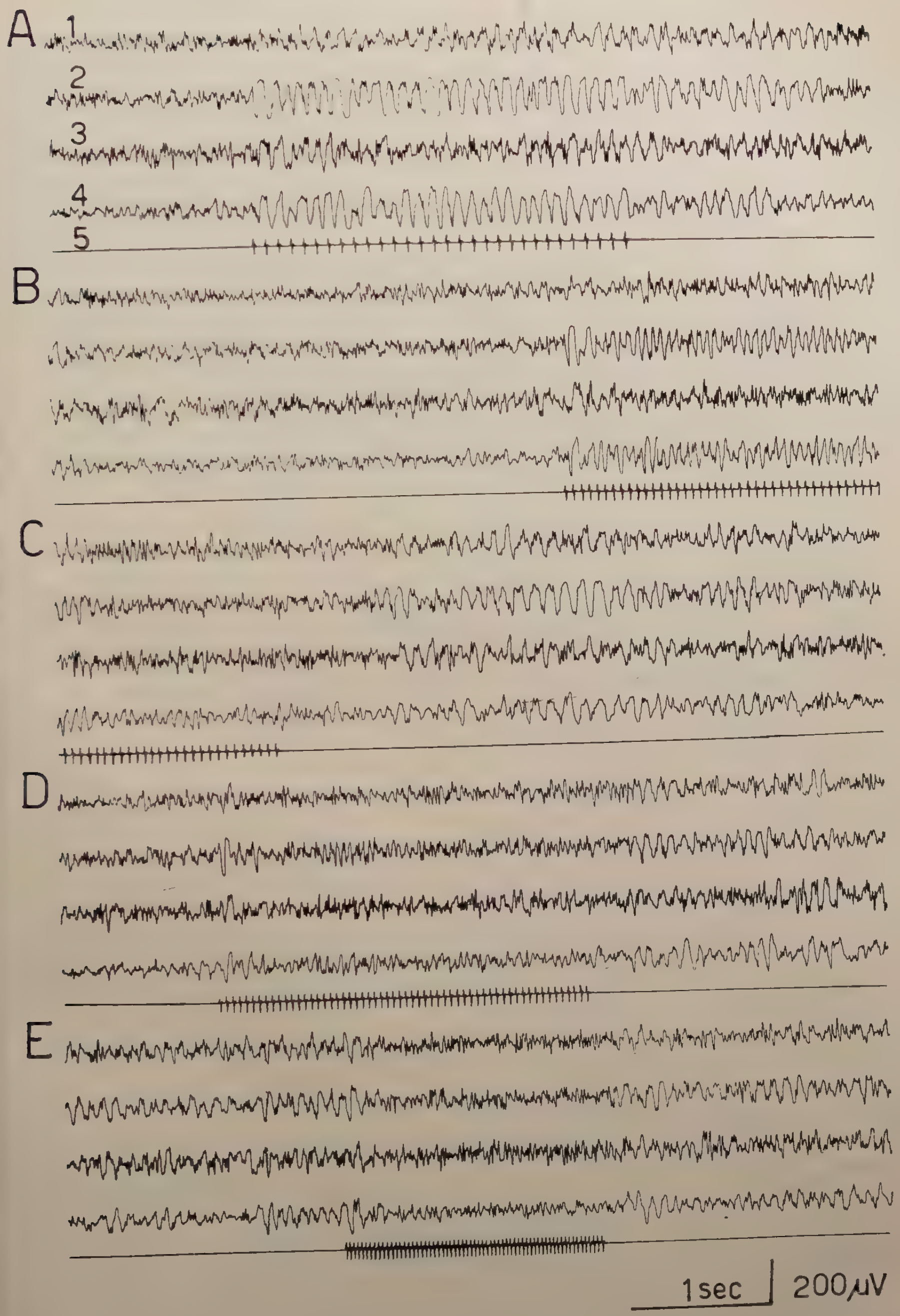


Fig. 6.

of electrical pulses (6-10/sec) will still synchronize the EEG. The pulse rate at which stimulation of the medullary structures starts to elicit arousal is not very critical but has a wide range sometimes extending rather higher than would be anticipated.

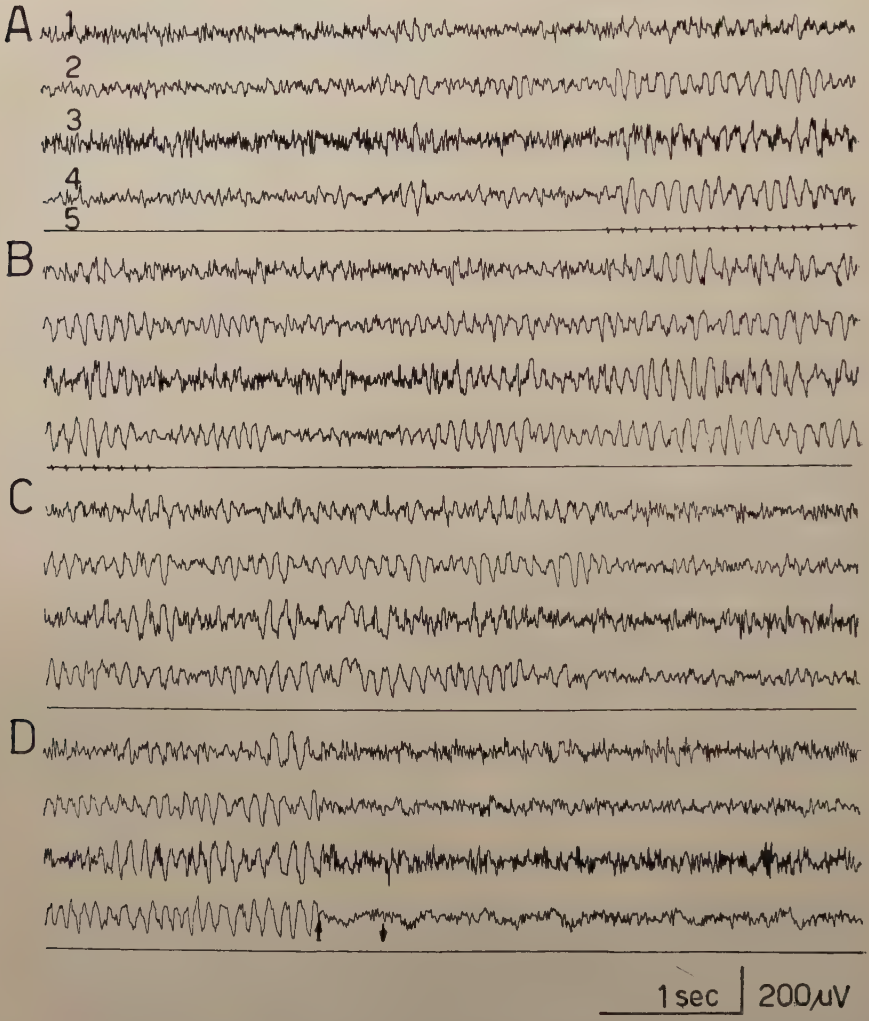


Fig. 7. – *EEG synchronization outlasting stimulation of region of solitary tract.*

Same cat as Fig. 3 (voltage and pulse duration same). Continuous record from above downwards. Synchronization produced by stimulation at 10/sec in *A* and *B* is followed by long lasting after-effect (B, C and D). Acoustic stimulation, between arrows in *D* (whistle), produces arousal.

4. *EEG synchronization outlasting the period of stimulation.* — A frequent finding, which will be returned to in more detail in the discussion, was the phenomen of EEG synchronization continuing for variable periods of time after terminating the electrical stimulation of the medullary structures. Whereas in some experiments synchronization ceased abruptly as soon as the stimulus was turned off, (e. g., Fig. 2 *B*) in others spindling went on intermittently. The rhythm of this after-effect was always the same as that of the inherent EEG rhythm. Persistance of synchronization beyond the period of stimulation may be seen in Figs. 3 *A*, *D* and 7 *B-D*. It will be noted that even after stimulation at 18/sec (Fig. 6 *B-C*) and at 20/sec (Fig. 6 *D*), although the fast rate of electrical pulses did not elicit synchronization during the period of stimulation, some after-effect was still apparent. It should be emphasized that reference is being made to instances in which synchronization was induced on a background of arousal or of moderate synchronization. The after-effect is particularly evident in Fig. 7, where the synchronous rhythms outlasted the end of the stimulation for 17 seconds, and were disrupted only when a startling auditory stimulus was applied (Fig. 7 *D*). It appears therefore that electrical stimulation of the region of the nucleus of the solitary tract is not only capable of driving the synchronizing mechanisms temporarily for the duration of stimulation, but that a more prolonged shift in the background of activity towards " drowsiness " may also take place.

5. *Desynchronization of the EEG produced by low frequency stimulation of the lower brain stem.* — As mentioned in the first section of the Results, in a number of experiments points were found in the medulla which upon stimulation at low frequency elicited desynchronization of the EEG. This rather surprising finding is illustrated in Figs. 9 and 10. It will be noted (Fig. 9) that stimulation with frequencies as low as 1, 2, 5 and 10/sec are capable of eliciting arousal. Fig. 10 is another example of this finding, and it also shows the close anatomical proximity of the medullary points eliciting EEG synchronization and EEG arousal. It was sufficient to move the stimulating electrode 1 mm more ventrally so as to have arousal give way to synchronization (Fig. 10 *B*). The structures seem to be so closely intermingled that at times one is without doubt stimulating both simultaneously. In Fig. 10 *C*, for instance, synchronization seems to predominate over arousal during the period of stimu-

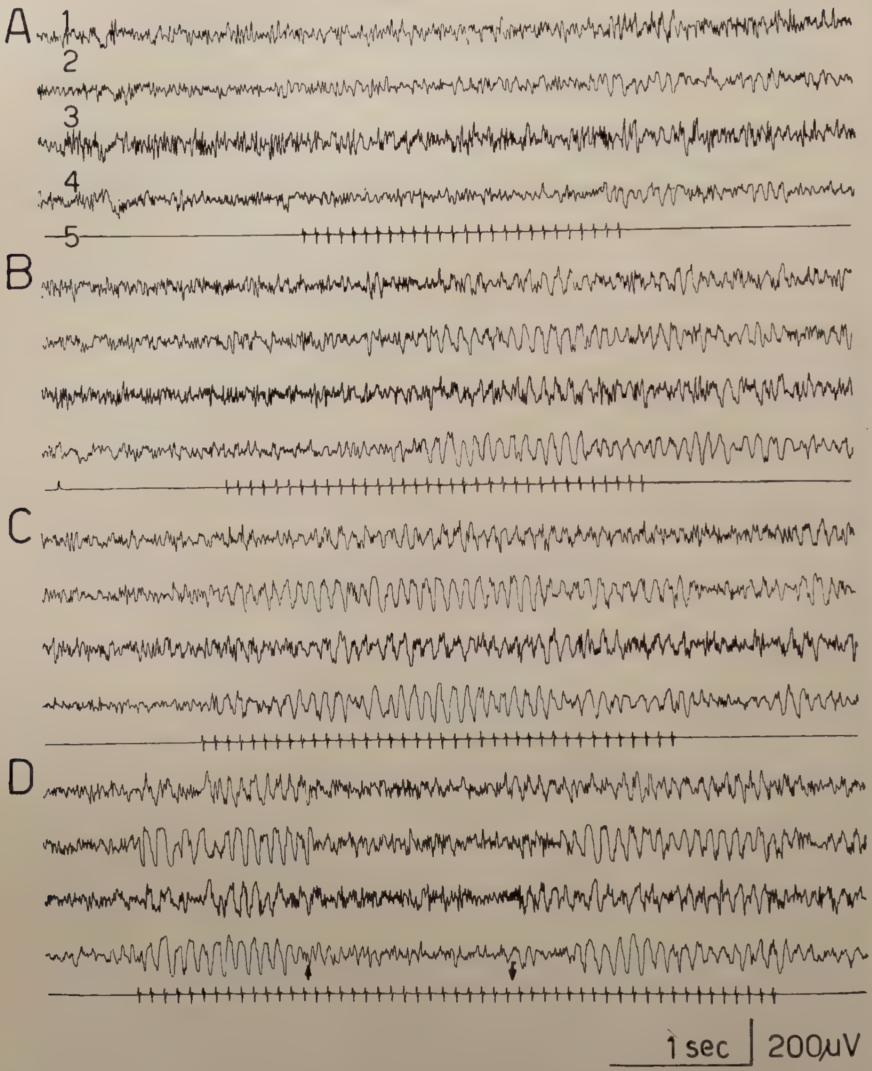


Fig. 8. – *Influence of EEG background on response.*

Same cat as Fig. 3 (voltage and pulse duration same). Effect of acoustic stimulation (arrows) on synchronization produced by stimulation at 11/sec of nucleus solitarius. *A*: 10 sec after acoustic stimulus (whistle) of Fig. 7 D. Medullary stimulation does not elicit synchronization. *B*: 90 sec after A. Some synchronization occurs during latter part of electrical stimulation. *C*: 10 sec after *B*. Complete recovery of the response. *D*: 10 sec after *C*. Acoustic stimulation (arrows) during electrical stimulation abolishes synchronization.

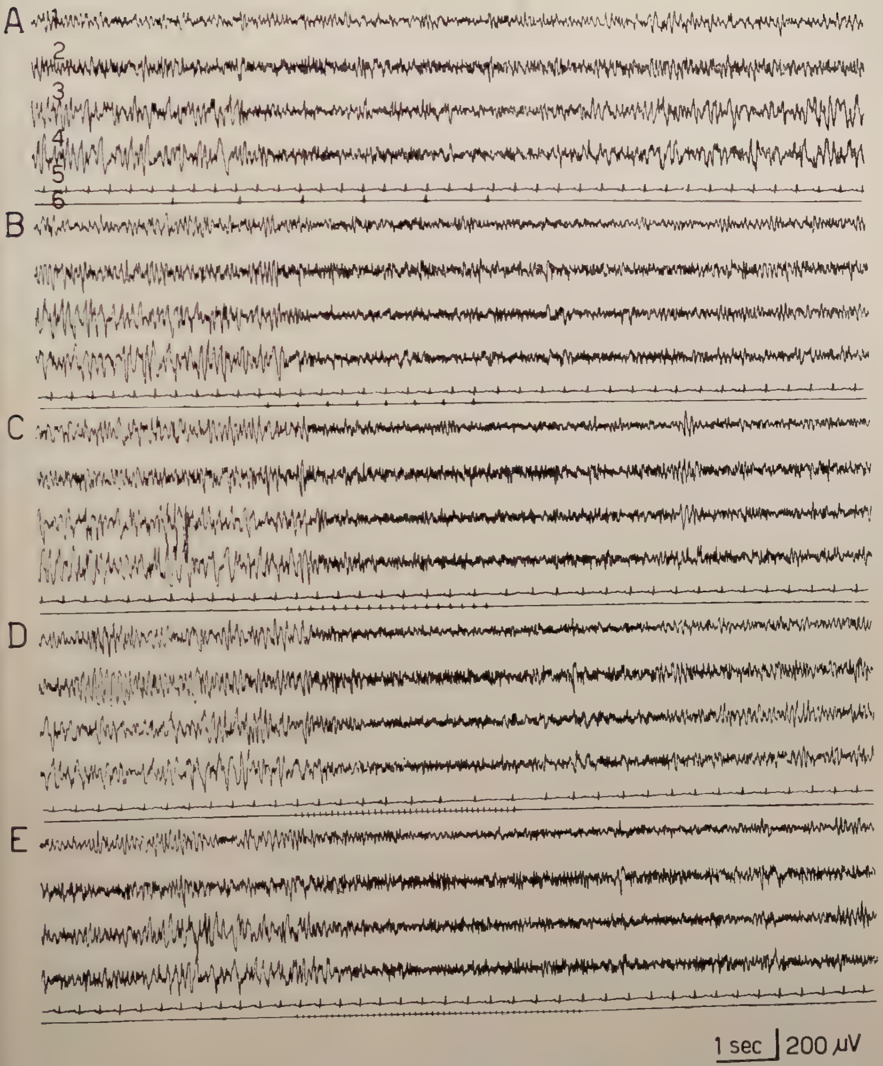


Fig. 9. – *EEG arousal and bradycardia elicited by low rate stimulation of region of solitary tract.*

*Encéphale isolé* cat. *Bipolar records.* 1: right parieto-temporal; 2. right temporo-occipital; 3: left parieto-temporal; 4: left temporo-occipital; 5: EKG; 6: stimulus marker. Stimulation with rectangular pulses of 3 V, 3 msec in *A-D* and 1 msec in *E*. *A*: stimulation at 1/sec causes EEG arousal. (Stimulation with same voltage and frequency but at 1 msec pulse duration had no effect). *B*: stimulation at 2/sec causes EEG arousal. C: stimulation at 5/sec causes EEG arousal with slight bradycardia at the end of stimulation. *D*: stimulation at 10/sec causes EEG arousal and bradycardia. E: stimulation at 10/sec (1 msec pulse duration) causes EEG arousal, but without bradycardia.

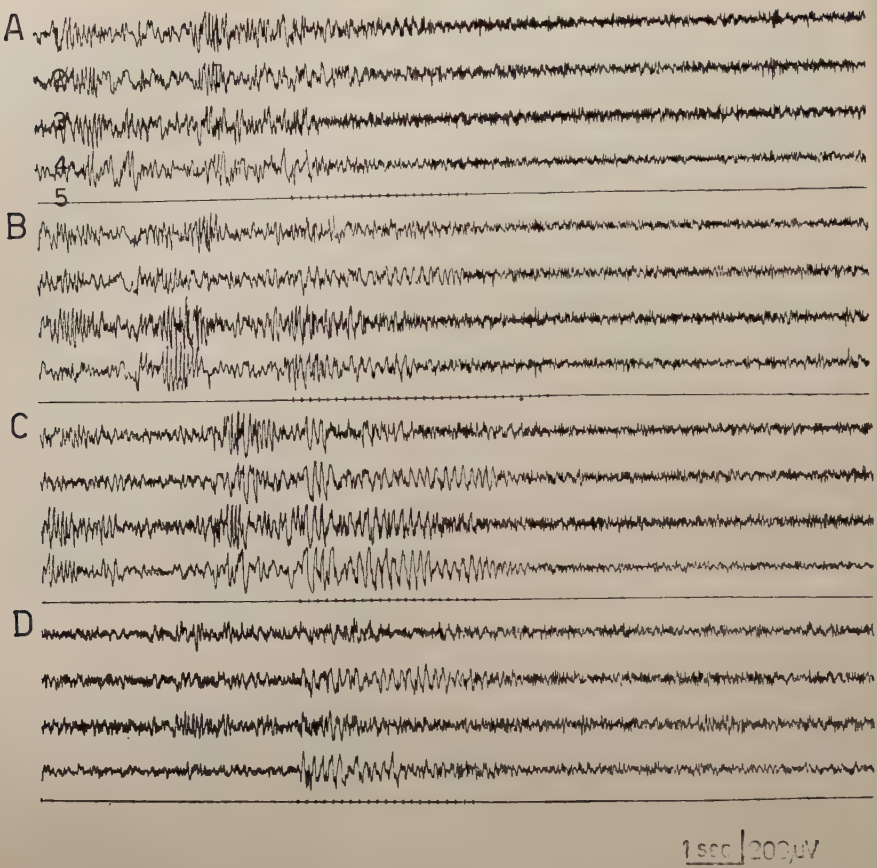


Fig. 10. – *Close proximity of medullary points eliciting synchronization and arousal at low rate stimulation.*

*Encéphale isolé* cat. Stimulation with rectangular pulses of 3 V, 3 msec, 6/sec. *Bipolar records.* 1: right parieto-temporal; 2: right temporo-occipital; 3: left parieto-temporal; 4: left temporo-occipital; 5: stimulus marker. *A*: stimulation at point 1,5 mm dorsally to the region of solitary tract elicits EEG arousal. *B*: during stimulation at point 1 mm more ventrally synchronization gives way to arousal. *C*: stimulation at point 0,5 mm ventrally to that in *B* (region of solitary tract) causes synchronization but arousal ensues immediately upon cessation of stimulation. *D*: stimulation at same point as in *C* on a background of arousal is less effective in producing synchronization. 5 sec interval between records *C* and *D*.

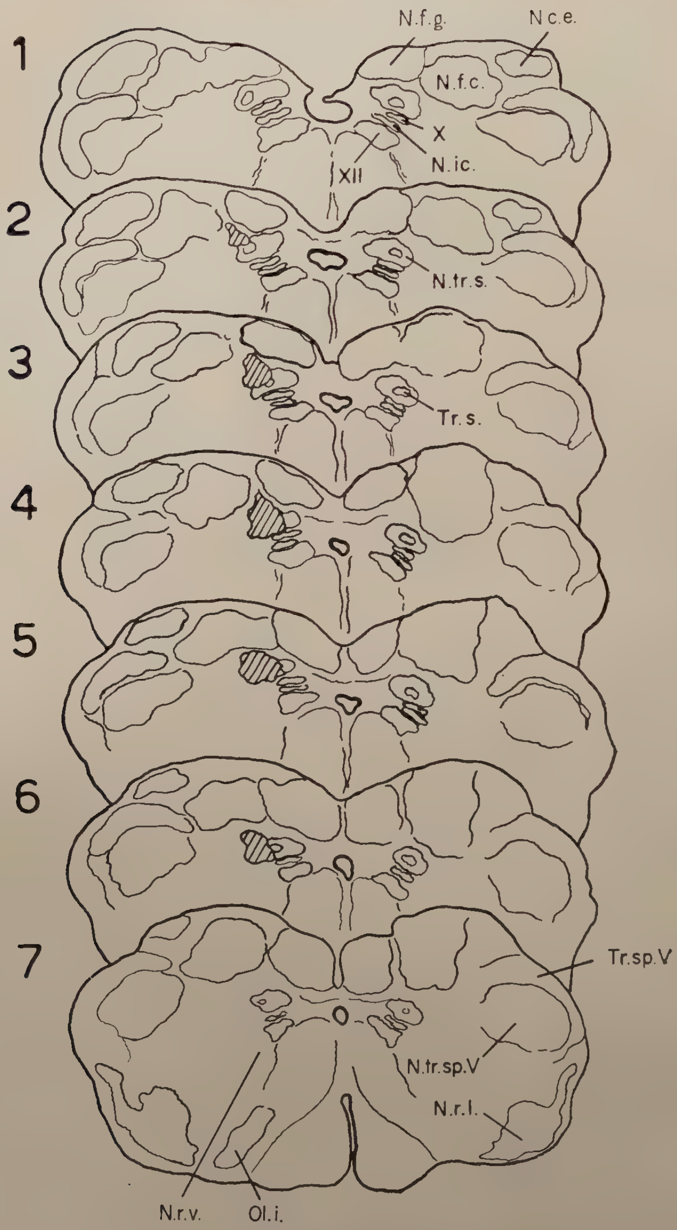
lation, but as soon as the stimulus was terminated arousal ensued, as though the neural mechanisms responsible for arousal had suddenly been liberated from a restraining influence.

All this would seem to indicate that EEG arousal does not necessarily require high rate stimulation of brain stem structures, but rather that if the localizing power of the stimulating electrode is good and the stimulus parameters carefully selected, arousal may be produced even with a stimulus frequency of 1 per second.

6. *Anatomico-physiological considerations concerning the medullary structures stimulated.* — Despite the difficulties of localization which are inherent in stimulation of the medulla, due to the close anatomical relationships of numerous nuclei and through fibers, we were able to dissociate a number of the responses by avoiding maximal and supramaximal intensities of stimulation, by careful selection of other stimulus parameters and by using electrodes of high localizing power. The localization of the EEG synchronizing structures was found to be critical, in that displacement of the electrode 1 mm dorsally or ventrally from a point eliciting synchronization caused the response to disappear completely. Upon moving the electrode back to its original position the response once more appeared. This is clearly illustrated in Fig. 2. Besides, as stated in the previous section of the Results, in some experiments the same low-rate stimulation parameters which elicited synchronization, yielded typical EEG arousal upon displacing the electrode 1 mm or less to other medullary loci in the same preparation.

This thesis is further born out by examination of the effects of medullary stimulation on heart rate (Fig. 9). The bradycardic effect of medullary stimulation could be readily dissociated from the arousal effect by careful variation of the stimulus parameters. Whereas stimulation at 2/sec produced only arousal, a frequency of 5/sec proved to be just threshold for the bradycardic effect. Stimulation at 10/sec in the same experiment gave clearcut bradycardia and arousal, but it was sufficient to cut down the pulse duration at this frequency from 3 to 1 msec to make the stimulus subthreshold for the bradycardic structures but still adequate to produce EEG arousal (Fig. 9 *C*).

With regard to circulatory effects produced by medullary stimulation we found that the points yielding EEG synchronization only infrequently gave bradycardia, which incidentally was never so intense as to produce a fall of systemic blood pressure (the vasomotor effects of medullary stimulation had been abolished by cervical transection). Even when the tip of our stimulating electrode was very close to the dorsal motor vagal nucleus, EEG synchronization


1
2
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Tr.s.
Tr.sp.V
N.tr.sp.V
N.r.l.
N.r.v.
Ol.i.

Fig. 11.

was observed without the slightest evidence of bradycardia. This finding may be explained by our knowledge of the topical localization of the cardiac center in the dorsal motor vagal nucleus (18) and by the high localizing power of our stimulating elecrtode which in these instances was probably close to that part of the vagal nucleus which is not concerned with regulation of heart rate. It would appear therefore that synchronization of the EEG is not due to stimulation of the terminals of Hering's and Cyon's nerves nor of the corresponding postsynaptic neurons, but rather to the excitation of nerve cells or fibers not belonging exclusively to the baroceptive system.

Synchronizing effects were actually obtained not only from the tractus solitarius and the nucleus of the tractus solitarius but also from the nucleus reticularis ventralis. In the same preparations in which positive results were obtained from these medullary points, the following structures were inactive when stimulated on the *same* background and with the *same* parameters of stimulation: nucleus fasciculi gracilis, nucleus fasciculi cuneati, nucleus reticularis paramedianus, bulbar pyramids, bulbar olivary complex, dorsal motor nucleus of vagus and hypoglossal nucleus. Fig. 11 gives in transverse section the extent of the lesion of the medullary area which when stimulated at low frequency produced synchronization in the experiment from which the records of Figs. 3, 5-8 were taken.

Another of the difficulties of localization was to satisfy ourselves that we were not co-stimulating the neurons of the dorsal column nuclei and the fibers of the medial lemniscus which lie just dorsally to the region of the nucleus solitarius. This problem was amenable to solution through the use of the primary responses in the contralateral somesthetic cortex as an index of lemniscal contamination. Fig. 12 *A-B* shows the primary responses sharply limited to the

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Fig. 11. – *Electrolytic localization of point stimulated in the experiment illustrated in Figs. 3, 5-8.*

The drawings correspond to histological sections taken at equal intervals of 360 $\mu$ and progressively numbered in rostro-caudal direction. The hatched areas correspond to the electrolytic lesion.

N.c.e.: external cuneate nucleus; N.f.c.: nucleus cuneatus; N.f.g.: nucleus gracilis; N.ic.: nucleus intercalatus; N.r.l.: lateral reticular nucleus; N.r.v.: nucleus reticularis ventralis; N.tr.s.: nucleus of solitary tract; N.tr. sp.V: spinal nucleus of trigeminal nerve; Ol.i.: inferior olive; Tr.s.: tractus solitarius; Tr. sp.V: spinal tract of trigeminal nerve; X: dorsal motor nucleus of vagus; XII: motor nucleus of XII nerve.

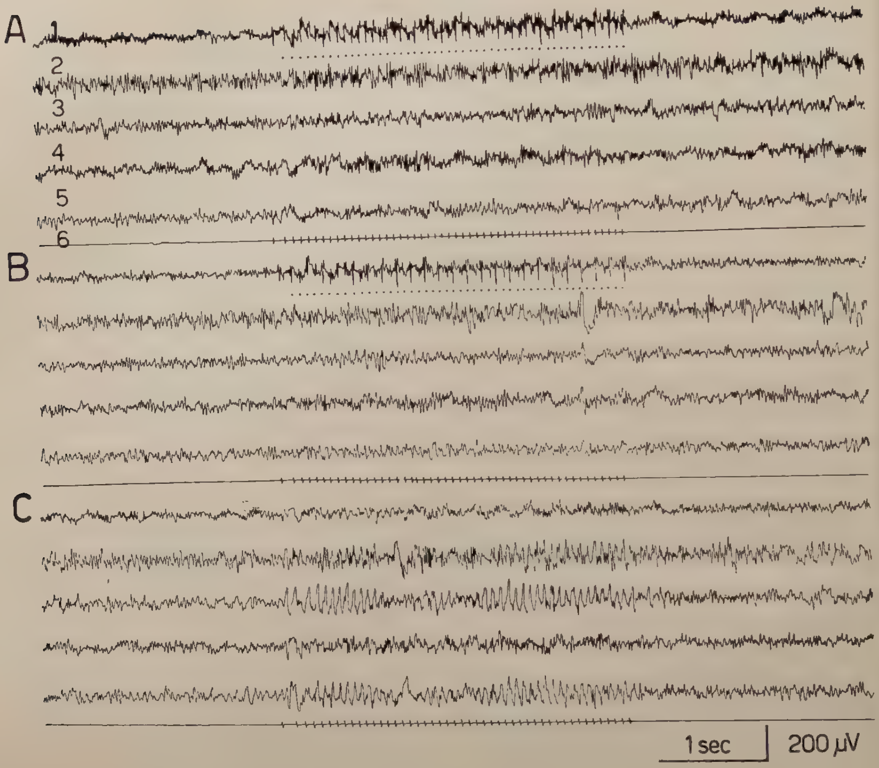

Fig. 12. – *Comparison of responses elicited by stimulation of nuclei of posterior column and region of solitary tract.*

*Encéphale isolé* cat. *Bipolar records.* 1: right fronto-frontal; 2: right parieto-temporal; 3: right occipito-occipital; 4: left fronto-frontal; 5: left occipito-occipital; 6: stimulus marker. Stimulation with rectangular pulses of 3 V, 3 msec, 15/sec. The inherent EEG rhythm of this cat was 15/sec during spontaneous synchronization. *A* and *B*: evoked responses in right frontal lead elicited by stimulation of nuclei of left posterior columns. C: synchronization in occipital leads (15/sec) elicited by stimulation of region of left tractus solitarius.

contralateral frontal area, while generalized EEG synchronization is absent. By gradually displacing the tip of the stimulating electrode dorso-ventrally or latero-medially, bilateral EEG synchronization was obtained without any trace of lemniscal response (Fig. 12 *C*). Thus, evidence is provided that EEG synchronization may be clearly dissociated from the effects of co-stimulation of the lemniscal system. Another possibility exists that the electrode placed in the region of the tractus solitarius co-stimulates passage fibers arising from

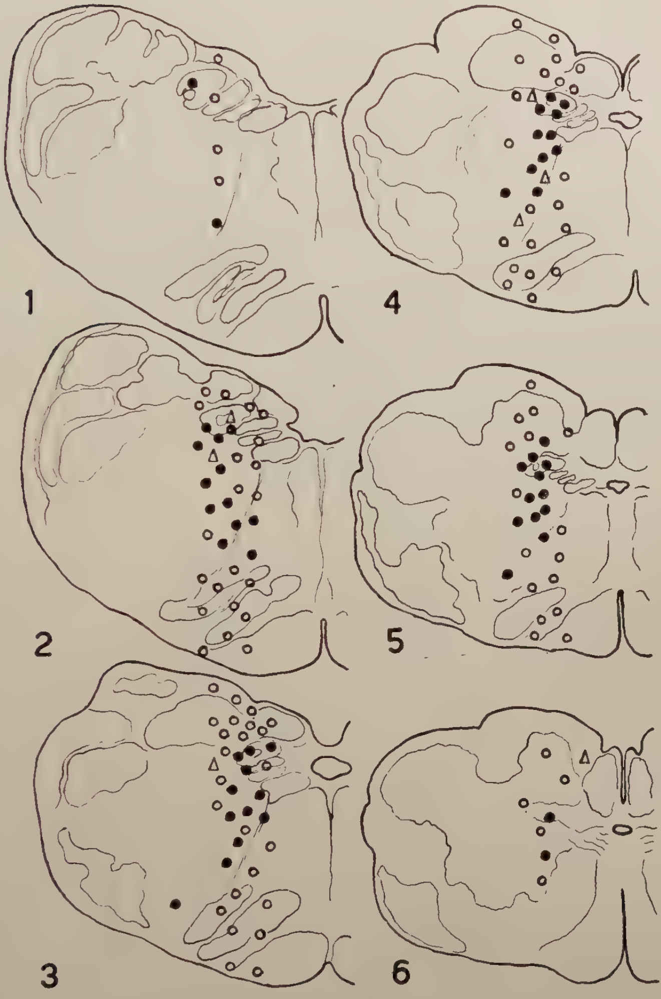

Fig. 13. – *Serial sections of the medulla taken at equal intervals and progressively numbered in rostro-caudal direction.*

On the left side of the drawings the stimulated points have been indicated. *Full circles*: points whose threshold stimulation at low frequencies (1-10/sec) produced EEG synchronization. *Empty triangles*: points whose stimulation at low and high frequencies produced EEG arousal. *Empty circles*: points whose stimulation at low frequency did not produce any EEG change. We have included negative points only from experiments in which synchronization could be elicited from another point. See Fig. 11 for anatomical landmarks.

the cerebellar fastigial nuclei. In particular Walberg, Pompeiano, Brodal and Jansen (48) have given anatomical evidence that fibers of the hook bundle of Russell end or pass through a group of small neurons packed just laterally to the nucleus solitarius. This structure has been called " nucleus parasolitarius ". Evidence against the possibility that we were co-stimulating such passage fibers was provided by one experiment with a chronically (9 days) decerebellate cat in which the synchronizing responses were obtained as usual. Fig. 13, which consists of serial sections of the medulla, summarizes in composite form the medullary localities stimulated and the responses elicited.

7. *Attempts at identifying the pathways mediating the synchronizing responses.* — In an attempt to identify the pathways mediating

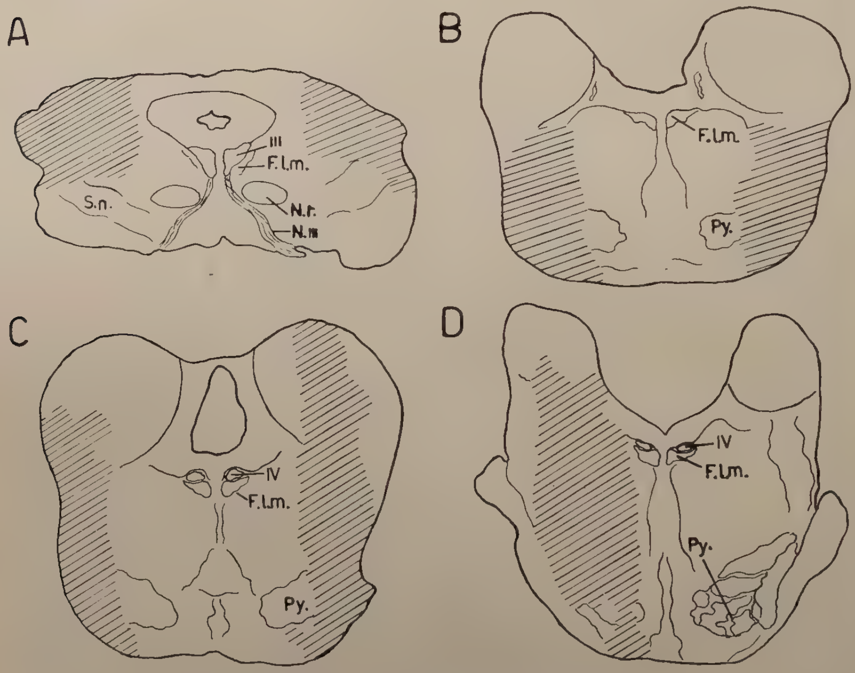

Fig. 14. – *Drawings of electrolytic lesions of brain stem.*

*A*, *B*: topography of lesion at different levels of the brain stem in the same preparation. *C*: topography of lesion of the brain stem in a different preparation. After these electrolytic lesions which spared the medial part of the brain stem, synchronization could still be elicited by stimulating the medullary synchronizing structures. *D*: electrolytic hemisection of the brain stem (right side) at the level of the trochlear nucleus in another preparation. See records of Fig. 15 concerning the physiological findings.

the synchronizing effect, several experiments were carried out in which acute or chronic partial lesions of the brain stem were made electrolytically. The following results are typical of those obtained

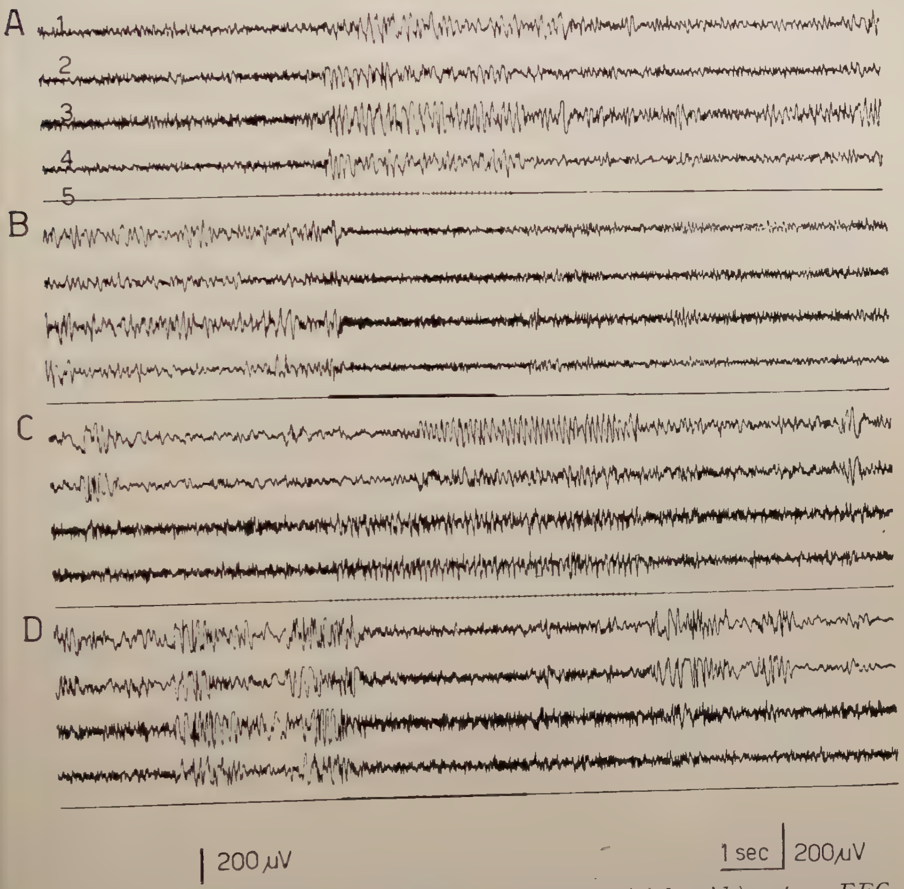

Fig. 15. – *Effect of hemisection of brainstem (right side) upon EEG synchronization and arousal elicited by stimulation of the medullary reticular formation.*

*Encéphale isolé* cat. *Bipolar records.* 1: right parieto-temporal; 2: right temporo-occipital; 3: left parieto-temporal; 4: left temporo-occipital; 5: stimulus marker. Calibration of voltage on the left side is related to *A* and *B*, that on the right side is related to *C* and *D*. Stimulation of right nucleus reticularis ventralis. *A*, *B*: *before hemisection*. *A*: synchronization elicited by stimulation at 10/sec, 3 msec, 1.4 V. *B*: arousal elicited by stimulation at 300/sec, 1 msec, 1 V. *C*, *D*: *44 min after hemisection of brain stem ipsilaterally to side of stimulation.* (See Fig. 14 D). *C*: bilateral synchronization elicited by stimulation at 10/sec, 3 msec, 3 V. Note delay in appearance of responses on side of lesion. *D*: bilateral arousal elicited by stimulation at 300/sec, 1 msec, 2 V. Note earlier reappearance of spontaneous spindling on side ipsilateral to section.

in this group. Fig. 14 shows the topography of acute bilateral electrolytic lesions of the lateral brain stem in 2 cats (*A*, *B* and *C*) and a complete hemisection in another (*D*). It will be noted that in the first group of lesions the medial part of the brain stem was spared. After these operations, synchronization could still be elicited by stimulation of the medullary synchronizing structures. Fig. 15 illustrates the results obtained after complete hemisection of the brain stem at rostro-pontine level. Though there is some variation in the spontaneous EEG pattern, which was to be expected, and in the latency of the appearance of the synchronizing response, the latter was still clearly present bilaterally. The bilaterality was present when the hemisection was made either ipsilaterally or contralaterally to the stimulated side.

Though further experiments are required in this direction, with the data in hand at present it is clear that the synchronizing effects are not mediated by the lateral lemnisci and that they probably do not require the presence of the lateral part of the brain-stem reticular formation. The results of the experiments of hemisection suggest that the ascending pathways mediating the synchronized reponse may cross both above and below the transection. Whether the synchronization is mediated by some specialized portion of the ascending reticular system or by specific pathways running in the medial portion of the brain stem cannot be established as yet.

## Discussion

The purpose of the experiments undertaken in this investigation was to try and provide more direct evidence for the existence in the lower brain stem of EEG synchronizing structures. Though some progress has been made in this direction there are a number of problems which arise to which we do not have the answers but can only venture some speculation.

1. *Anatomical problems.* — Our effects were obtained by stimulating the region of the nucleus of the solitary tract and the underlying reticular formation (nucleus reticularis ventralis of the medulla). The first point to be discussed is whether our effects were due to stimulation of somata lying in these areas of the medulla or of fibers ending upon or arising from these nerve cells. The main difficulty in experiments of stimulation of the brain stem is repre-

sented by the possibility of co-stimulating through fibers or neighboring axons belonging to different systems. Control experiments have shown that our synchronizing effects cannot be explained as being due to co-stimulation i) of internal arcuate fibers arising from the posterior column nuclei and passing over or through the region explored to the medial lemniscus nor ii) of fastigiofugal fibers ending or crossing the region of the nucleus parasolitarius. Even if other possibilities of contamination cannot be disregarded, there is little doubt that these controls dispose of the main sources of error.

We believe therefore that our results are likely to be due to stimulation of somata lying in these medullary areas or of fibers ending upon them. The anatomical data pertaining to this part of our discussion have been reviewed elsewhere (35). For the purposes of the present discussion suffice it to say that the evidence in hand does not point to the synchronizing system of the lower brain stem as being an integral part of the system which influences cardiac rate. In most of our experiments synchronization was obtained without bradycardia, whereas slowing of the EEG obtained by stimulation of the baroceptors is always accompanied by bradycardia. This could be explained by assuming that separate fibers or separate collaterals are involved in the transmission of impulses from the baroceptor zones to the cardioinhibitory center and to the EEG synchronizing structures of the medulla. The physiological stimulus of raising intrasinusal pressure would always co-stimulate both these private paths, while a selective stimulation would be possible when electrical pulses are applied to well localized districts of the medulla. If our effects were obtained by postsynaptic stimulation of second order sensory neurons, it would be necessary to postulate that the nerve cells in the nucleus solitarius which mediate synchronization are different, and differently located, from those involved in cardiac inhibition. An alternative explanation would be that we were not stimulating nerve cells of the sensory nucleus of the glosso-pharyngeal nerve, but simply EEG synchronizing structures, neighboring to, probably anatomically related with (cfr. 12, 1, 45), but different from the thin column of nerve cells constituting the nucleus solitarius. It should also be recalled here that stimulation of the neurons of the nucleus reticularis ventralis of the medulla, which is not considered a part of the baroceptor-cardioinhibitory reflex arc, also produced EEG synchronization.

The third problem concerns the ascending pathways mediating the EEG synchronizing effects elicited by medullary stimulation. We have seen that our responses were still present when the lateral part of the brain stem had been interrupted on both sides at pontine or mesencephalic levels.

These findings fit satisfactorily with the anatomical data concerning the ascending projections from the bulbar reticular formation. In fact it has been clearly demonstrated (9) that most of the long ascending fibres of the reticular formation arise from the medial two thirds of the brain stem, and that actually some of them are given off by the nucleus reticularis ventralis of the medulla, namely from the ventro-medial region explored in our experiments.

There is not full agreement concerning the ascending projections from the gray matter of the solitary tract. Contrary to the old descriptions of Wallenberg (49), Allen (1, 2) and Krause (32), recent observations made on rats by Hawkes (19) with the Nauta method seem to indicate that the ascending fibres from the nucleus solitarius do not reach the diencephalic structures directly by following the course of the medial lemniscus, but they probably travel in the reticular system. According to Hawkes, degeneration in various districts of the periventricular system, and especially in the laterodorsal and dorsal tegmental nuclei, was also a constant finding in all animals having dorsal medullary lesions. However, it is not well known to what extent these systems of fibers arise from the nucleus solitarius or from the surrounding structures of the medulla affected by the lesion [1].

The last anatomical problem is represented by the fact that according to Favale, Loeb, Rossi and Sacco (15, 16) EEG synchronization can be produced in the cat by stimulating widespread areas of the reticular formation. In our experiments the points giving this effect were rather strictly delimited and intermingled with others giving only EEG arousal, occasionally even as a response to low rate stimulation. The reasons for this discrepancy are probably of methodological character. Favale, Loeb, Rossi and Sacco worked with electrodes implanted in free-moving animals, so that the tips of the stimulating electrodes could not be displaced millimeter by millimeter as in our preparations; clear-cut evidence of localization can be obtained only when a given response is obtained, lost and later obtained again by raising or lowering the electrodes in the same animal, and in a short period of time. On the other hand we did not explore the pons, the midbrain, nor even the lateral part of the medulla. Consequently we are unable to state at the present what our method might give if employed in the huge task of systematic exploration of the entire brain stem. In a few experiments we obtained different patterns of EEG synchronization from rather extensive areas of the midbrain tegmentum, in agreement with previous authors (see Introduction for references). One should not forget that stimulation of the rostral part of the brain stem may involve excitation of axons ascending from lower lying structures of the cerebello-thalamic pathways.

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[1] The existence of a "vago-hypothalamic pathway" arising from the dorsal vagal nucleus, postulated by anatomical (41, 47) and physiological studies (24, 10), is disproved by the recent observations of Mitchell and Wardick (37) and Hawkes (19). Therefore it has not been considered in our discussion.

2. *Physiological problems.* — With regard to the effects of changing stimulation frequency some discussion is necessary. It was mentioned briefly in the presentation of the results that synchronization of the EEG which is elicited by low-frequency stimulation of the region of the nucleus of the solitary tract, differs in a number of respects from the EEG changes obtained by stimulating the midline nuclei of the thalamus (see 27, 29). Our effect is similar to that produced by thalamic stimulation in that i) both are bilaterally distributed on the cortex, ii) the optimum rate of stimulation is approximately that of the spontaneous rhythm, iii) there is a waxing and waning of the synchronous waves and iv) spindles can be tripped by single shocks. The dissimilarities, however, deserve consideration.

i) With stimulation of the bulbar structures there was generally no evidence of recruitment of the cortical neurons, even when the electrical pulses were barely supraliminar for the EEG effect.

ii) The frequency of the induced cortical waves was independent of the rate of medullary stimulation when the latter did not exceed the inherent spindling rate of the EEG.

iii) The slow cortical waves, induced by bulbar stimulation, frequently outlasted the end of the stimulus for many seconds and even minutes.

iv) Whereas during thalamic stimulation recruitment appears preferentially in the frontal areas of the cortex, during medullary stimulation synchronization was more easily obtained in the occipital and parieto-temporal regions.

v) According to Jasper (28) the spindles, tripped by single shocks applied to the midline nuclei of the thalamus, last for a number of seconds, and the latency of the effect in the occipital region may be of the order of one second. The response to single shocks observed in our experiments was composed of 3 or 4 waves only which came on almost immediately after delivering the stimulus. These differences, however, may be related to the location of the thalamic and brain stem electrodes or to the background of EEG activity.

The observation that low frequency stimulation of certain points in the medulla elicited EEG arousal, whereas other points in close proximity gave synchronization, comes now under discussion. Favale, Loeb, Rossi and Sacco (16) reported a similar observation by stimulating at 5/sec different points of the pontine reticular formation in the intact animal. The possibility that the effect may be due to adrenaline discharge elicited by co-stimulation of descending fibers cannot be accepted for *encéphale isolé* cats. There is little doubt however that reticular arousal is normally elicited by high rate stimulation, even in the unanesthetized animal. Our observations of EEG arousal elicited by low rate stimulation were limited to the region of the solitary tract, and might be explained by stimulation of presynaptic fibers or of postsynaptic neurons related to the chemoceptive system, which exerts a strong arousing influence on the EEG (25). Should these observations be extended to other brain stem regions of the *encéphale isolé*, in confirmation of the findings on the intact animal (15, 16), the hypothesis of stimula tion of ascending nociceptive fibers should be considered (see 20). Before the difficulties related with co-stimulation are solved, it would be an unwarranted hypothesis to suggest that there are in the reticular activating system a group of neurons which may give EEG arousal also when stimulated with low rate pulses.

A distinction between brain stem structures yielding respectively EEG activation and EEG synchronization would appear more justified, at the present state of our knowledge. It is true that even this hypothesis does not remain unchallenged. The observation so frequently made in both behavioral (11, 21, 13, 43, 42, 15, 16) and EEG studies (44, 17, 13, 3, 26, 33, 40, 39, 15, 16, 46, 34) that stimulation of sleep inducing and/or EEG synchronizing structures at high frequencies elicits arousal would suggest the opposite point of view, namely the existence of one system only, reacting differently in accordance with the frequency of stimulation. It may however be argued that failing extremely accurate localization of the points stimulated, high frequency stimulation may produce EEG arousal by driving, through temporal summation, neurons whose axons or somata are probably scattered among or closely neighboring to the cells of the antagonistic system.

From the data relating to the influence of the EEG background on the synchronizing response, it would appear that there are two mutually antagonistic systems at work, one synchronizing and the other desynchronizing. Between extreme synchronization and extreme desynchronization lies an area of overlapping influence of both systems in which neither has the upper hand. This is a state of delicate balance characterized by intermittent phases of near-arousal and slight synchronization as evidenced by the EEG. Any slight change in the internal or external environment is likely to tip the balance. At the two extremes we find areas where the arousal or synchronization are so strong that no amount of the opposite influence can change the pattern. These may be considered as abnormal situations, e. g., the deep synchronization produced by large doses of a barbiturate or the strong arousal pattern seen after adrenaline administration. In between these two extremes, and on either side of the area of overlap of the synchronizing and desynchronizing influences, there are two wide regions of synchronization and desynchronization in which the pattern may be shifted in either direction depending on the amount of desynchronizing or synchronizing influence which is exerted on the system.

There are two main hypotheses which might be advanced to explain the mechanism of synchronization elicited by stimulating given regions of the lower brain stem: *a*) reciprocal inhibition of the brain stem activating system leading to a release of the diencephalic pacemaker; *b*) direct excitation of EEG synchronizing structures of the diencephalon, mainly of the thalamus (see 28) and of the nucleus caudatus (see 30).

In favour of the first hypothesis is the observation that EEG synchronization may appreciably outlast the end of the bulbar stimulation. This is also the most likely explanation of the behavioral sleep observed by Hess (21) and the EEG changes reported by Caspers and Winkel (13), which appeared only after application to the midbrain of several prolonged volleys of pulses. It is interesting in this respect that Favale, Loeb, Rossi and Sacco (16) obtained behavioral signs of sleep only in those cases in which the EEG synchronization greatly outlasted the stimulus. On the other hand this hypothesis would hardly explain the onset of EEG synchronization so soon after the first electric pulse is applied to the medulla. Furthermore, the observation that the frequency of the synchronous waves may increase when stimulation rate exceeds the inherent EEG rate, speaks in favour of a more direct effect on the thalamic centers.

Obviously these two hypotheses are not mutually exclusive In fact the best way to explain our results would be if the two mechanisms were working hand in hand.

## SUMMARY

1. In acute *encéphale isolé* cats, low frequency electrical stimulation (1-16/sec) in the region of the nucleus of the solitary tract and of nucleus reticularis ventralis, resulted in synchronization of the EEG. The resultant pattern of electrical activity was one of large amplitude slow waves distributed bilaterally in frontal, parietal, temporal and occipital leads.

2. These synchronizing effects were most easily elicited when the endogenous background of the EEG was not fully desynchronized. When intense activation was present spontaneously, or was induced by sensory stimulation, these effects could not be elicited or were abolished.

3. In most cases the repetition rate of the EEG waves during elicited synchronization was identical with that of the inherent EEG rhythm. However, when the frequency of stimulation exceeded the autogenous spindling rate, the frequency of the elicited waves was apt to follow the rate of stimulation. EEG activation ensued almost constantly when the pulse rate was higher than 30/sec.

4. In the main, synchronization of the EEG persisted for varying periods of time subsequent to the end of stimulation. In some cases it ceased abruptly as soon as stimulation was over.

5. Low frequency stimulation (1-10/sec) of neighboring points in the same region of the medulla was found to produce EEG arousal.

6. It has been established that the results with regard to synchronization of the EEG are not due to stimulation of the posterior column nuclei or fibers of the medial lemniscus, nor of fastigiobulbar fibers running closely to the region of the solitary tract.

7. Experiments performed on cats with acute or chronic lesions of the brain stem indicate that induced EEG synchronization may be obtained when only the medial part of the pons or of the midbrain are spared by the lesion.

8. The relation of these findings to the more general problem of the possible functions of the brain stem synchronizing structures is discussed.

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# ON THE MECHANISM OF THE TACHYCARDIA PRODUCED BY ELECTRICAL STIMULATION OF THE POSTERIOR HYPOTHALAMUS[1]

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## INTRODUCTION

Since the first studies on the general effects, and particularly on the cardiovascular effects, of hypothalamic stimulation, it has been clear that the systemic arterial hypertension, which occurs when the postero-lateral areas of the hypothalamus are stimulated electrically, is regularly accompanied by an increase in the heart-rate (see 11, 18, 7, 8).

Although the tachycardiac response appears in every case, nevertheless, according to those authors who are particularly interested in it, it is, at least in the cat, moderate in amount (16, 21) in spite of the marked activation of the cardiac nerves demonstrated by Pitts, Larrabee and Bronk (16). The latter authors attribute the small size of the tachycardiac response to the fact that their animals spontaneously showed a heart-rate which was already rather high as a result, they thought, of the low vagal tone. This interpretation is supported by the observations of van Bogaert (1, 2) and of Rushmer *et al.* (19), who, working with the dog, an animal which normally shows high vagal tone, have observed tachycardiac responses of considerable intensity which could, moreover, be preceded by a short period of bradycardia or of brady-arrhytmia (1, 2).

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[1] English translation by T. D. M. Roberts and J. D. Christie (Glasgow).

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The mechanism by which the hypothalamic tachycardia arises, cannot be explained solely in terms of adrenergic nervous factors, that is, in terms of the activation of those sympathetic fibres that have a chronotropic effect on the heart (also shown to occur, 16); for, in fact, the tachycardia is intensified at about 20 sec after the beginning of the stimulation; it is diminished but not abolished by cardiac sympathectomy; and it is cut short by adrenalectomy (21). Undoubtedly, if the increase in frequency of the heart-beat develops slowly in relation to the beginning of stimulation, a good part at least of the effect could be due to the discharge into the circulation of hormones from the suprarenal medulla, a phenomenon by now well documented.

In contradistinction to the chronotropic effects produced by hypothalamic stimulation, the intravenous administration of sympathomimetic substances, such as adrenaline and nor-adrenaline, produces, as well as marked hypertension, a quite clear and often intense bradycardia. It has been an established principle for many years that this slowing of the heart-beats is essentially a reflex phenomenon (9). Indeed, any increase in systemic pressure, either pharmacologically induced by introduction of hypertensive hormones into the circulation, or neurogenic, such as that produced by stimulation of the splanchnic nerves, increases the frequency of the discharge from the baroreceptors of the aorta and of the carotid sinus, and, by these pathways, produces an activation of the bulbar cardio-inhibitor centre. Actually, in the experiments carried out in this Institute over a number of years, it has always turned out that, at least in the dog, it is sufficient to leave intact only one of the buffer nerves in order to obtain a bradycardia by administration of adrenaline or by stimulation of the splanchnic nerves (15).

When we saw in the cat an intense tachycardiac reaction during experiments on stimulation of the posterior hypothalamus, we asked ourselves how such a tachycardia could come about if the animal had intact all four depressor nerves which could not fail to be activated by the considerable pressure rise.

We therefore thought it probable that the hypothalamic regulation of the heart-rate acts not only through a mechanism of activation of the sympathetico-adrenal system, but also through the intervention of other mechanisms not so far taken into account, one of which could consist of a simultaneous inhibition of the bulbar centre of the vagus.

The express intention of the present work is to examine this hypothesis.

## METHODS

The experiments were carried out on 15 adult cats of various weights between 2.5 and 4 kg, anaesthetised with nembutal (30 mg/kg by the intraperitoneal route).

The postero-lateral areas of the hypothalamus were reached by the technique of Hess (6) and were stimulated using bipolar electrodes of the Hess type, insulated along their whole length except for a region of 0.5 mm at the tip. The stimulating current was provided by an electronic impulse-generator, with radio-frequency output, which gave square waves whose intensity, frequency and duration could be varied independently. In these experiments we have held to those parameters which, according to our previous experience and to that of other authors, reliably produce adequate cardiovascular responses. The voltage, therefore, was kept at 3-4 V, the frequency was 135 stimuli per second and the duration of each stimulus was 2 msec. The period of stimulation was 20-30 sec.

The systemic arterial pressure was recorded from a femoral artery cannulated with a polythene cannula connected to a Sanborn capacitance electromanometer, coupled to a Sanborn Twin Viso. The paper-speed was such as to give a clear record of changes in pressure together with an accurate measure of the heart-rate. In some experiments the electrocardiogram also was recorded, on the second channel of the recorder, picking up from suitably constructed needles inserted under the skin of the four limbs. In such experiments the paper-speed was increased ten times.

The femoral vein of the same side was also cannulated and connected to a venous drip apparatus by a two-way connector fitted with valves. It was thus possible to introduce into the blood-stream known amounts of drugs either drop by drop using one of the channels of the connector, or rapidly through the other channel.

The operative procedures carried out on the animals from time to time were: section of the spinal cord, removal of the stellate ganglia, and bilateral adrenalectomy. The spinal cord was cut, either at the level of $T_{4-5}$ or at $C_{7-8}$, with a spatula whose sharp edges had been rounded off, after removing the vertebral laminae and cutting the dura mater. We endeavoured to reduce to a minimum the operative shock to the animal, avoiding the effects of compression or of dehydration, or excessive haemorrhages. For the removal of the two stellate ganglia, the dorsal route was followed at the level of the first two costo-vertebral articulations. With this technique, damage to the parietal pleura (with consequent pneumothorax) did not occur; and thus the animal's respiration remained spontaneous throughout the experiment. Adrenalectomy was carried out bilaterally by the dorsal extraperitoneal route, the arteries and veins being ligated and the gland removed without injuring the splanchnic nerves to the abdominal viscera.

At the end of the experiment, the cranium was opened widely; the brain was removed entire, and was placed in 10% formalin for later macro- and microscopical study of the localisation of the region stimulated.

## Results

1. *Stimulation of the hypothalamus in the intact animal.* — In basal conditions the heart-rate in our animals usually ranged between 120 and 160 beats per minute. Stimulation of the posterior hypothalamus in such animals always produced a tachycardia, more or less marked, which usually represented an increase of 20-30% or more over the control value (Fig. 1). Even those few animals in which, at the beginning of the experiment, the frequency was already much higher, above 200 beats/min, one could observe a tachycardiac response of about 15-20%.

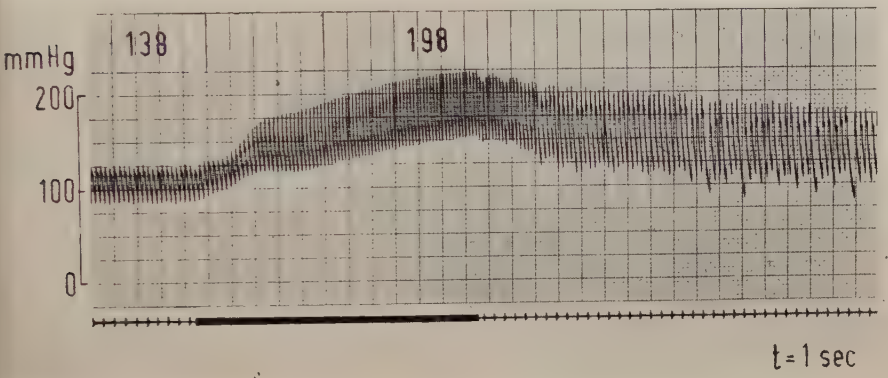

Fig. 1. – *Cardiovascular responses to electrical stimulation of the posterior hypothalamus.*

Cat under nembutal anaesthesia. Intact animal. Electro-manometric recording of the systemic arterial pressure. From above downwards: Heart-rate per minute calculated over a period of 10 sec, arterial pressure, time in sec; the continuous line indicates the period of stimulation.

As a result of the stimulation the heart-rate increases by 43.5%. At the end of the stimulation a prolonged bradycardia appears.

The increase in heart-rate already appeared in the first 10 seconds from the beginning of the stimulation, but the most intense effect was seen in the following seconds. The electrocardiographic recordings showed that the tachycardia was always of the sinus type (Fig. 2).

At the end of the period of stimulation, while the systemic arterial pressure began gradually to decline, one could see, at least

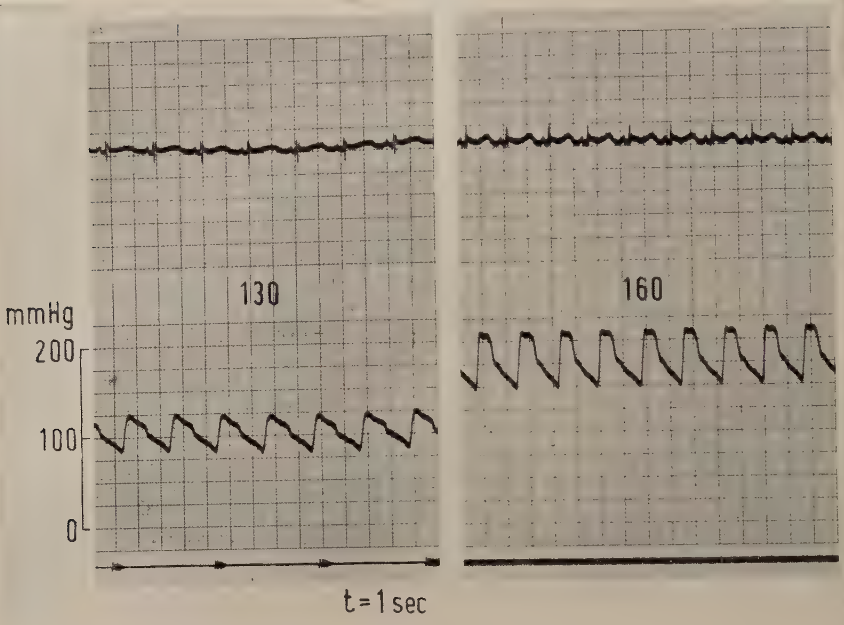

Fig. 2. – *Sinus tachycardia produced by electrical stimulation of the posterior hypothalamus.*

Intact cat. From above downwards: electrocardiogram, heart-rate, arterial pressure, time in sec. Left: record obtained before the beginning of the stimulation; right: during hypothalamic stimulation.

As a result of the stimulation the heart-rate increases by 23%.

in most of the experiments, a bradycardia accompanied by a temporary increase in the pulse-pressure. Only in a few animals was this bradycardia absent.

2. *Stimulation of the hypothalamus after section of spinal cord at thoracic level.* — Section of the spinal cord carried out at $T_{4-5}$, to preserve the sympathetic innervation of the heart, always produced a fall in blood-pressure, averaging about 20-40 mm Hg; the systolic pressure after the operation was of the order of 85-105 mm Hg. The heart-rate was not appreciably altered. Hypothalamic stimulation after the spinal section was not only still effective, but generally produced increases in pressure only a little less than those obtained in the intact animal; the tachycardiac response was still present in some cases practically unchanged from the controls, in most cases a fraction less (Fig. 3).

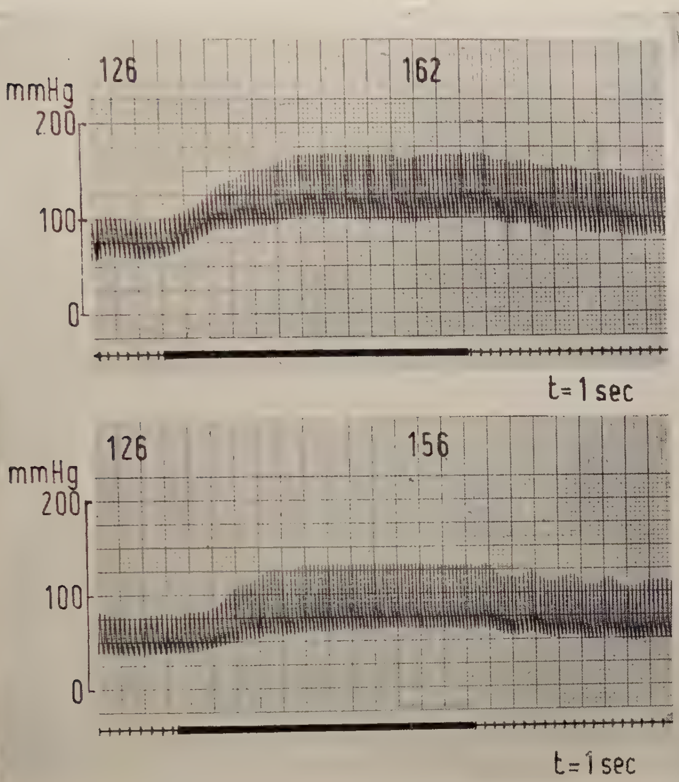

Fig. 3. – *Cardiovascular responses to electrical stimulation of the posterior hypothalamus after spinal section carried out at the level of* $T_{4\text{-}5}$.

The upper record was obtained in the intact animal, the lower record after spinal section. The increase in heart-rate, after the operation, is reduced from the 28.6% first obtained, to 23.8 %.

3. *Stimulation of the hypothalamus after bilateral removal of the stellate ganglia.* — Removal of the two stellate ganglia did not produce any significant change in the electromanometric record: in other words, one could not see changes of any consequence either in the level of the arterial pressure, or in the form of the pressure curve, or in the heart-rate. In these experimental conditions, stimulation of the hypothalamus produced a hypertensive effect exactly comparable with the effect obtained before removal of the stellate ganglia; the tachycardiac response was not abolished, but was less intense than that obtained before. From Fig. 4 one can see, indeed, that the arterial pressure rises equally in the presence and in the

absence of the stellate ganglia, although the response, in this case, starts with a latency of a few seconds. Calculation of the heart-rate, on the other hand, shows that, in the animal with the cardiac sympathetic supply removed, the increment in heart-rate is reduced from the 30% previously obtained, to 18%. The bradycardia which follows the cessation of the stimulation is not abolished by bilateral removal of the stellate ganglia.

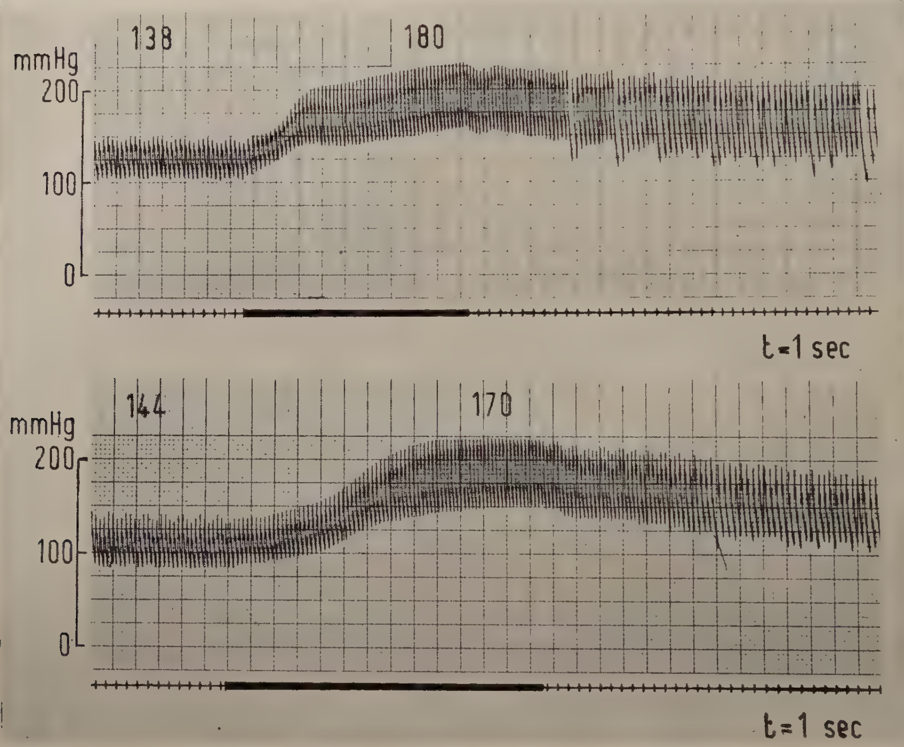

Fig. 4. – *Effects of bilateral removal of the stellate ganglia on the cardiovascular responses to electrical stimulation of the posterior hypothalamus.*

Upper record: intact animal; the heart-rate, as a result of the stimulation, increases by 30%; at the end of the stimulation an intense bradycardia appears.

Lower record: animal with stellate ganglia removed. The pressure rise begins with a delay of some seconds; the tachycardia is reduced to + 18 %; the post-stimulatory bradycardia is not abolished.

4. *Stimulation of the hypothalamus after bilateral adrenalectomy.* — Bilateral adrenalectomy also left the basal values of blood-pressure

and of heart-rate practically unchanged. The pressural responses to hypothalamic stimulation, before and after the operation, showed quantitatively similar behaviour. The tachycardia was slightly less intense; Fig. 5 shows the haemodynamic records obtained from the same animal before and after adrenalectomy. In this experiment, taking the average of six trials carried out with the same conditions of stimulation, we found that, with the adrenals intact, the increase in heart-rate was equal to 25% over the control values, while after removal of the glands, the increment was reduced to 20%.

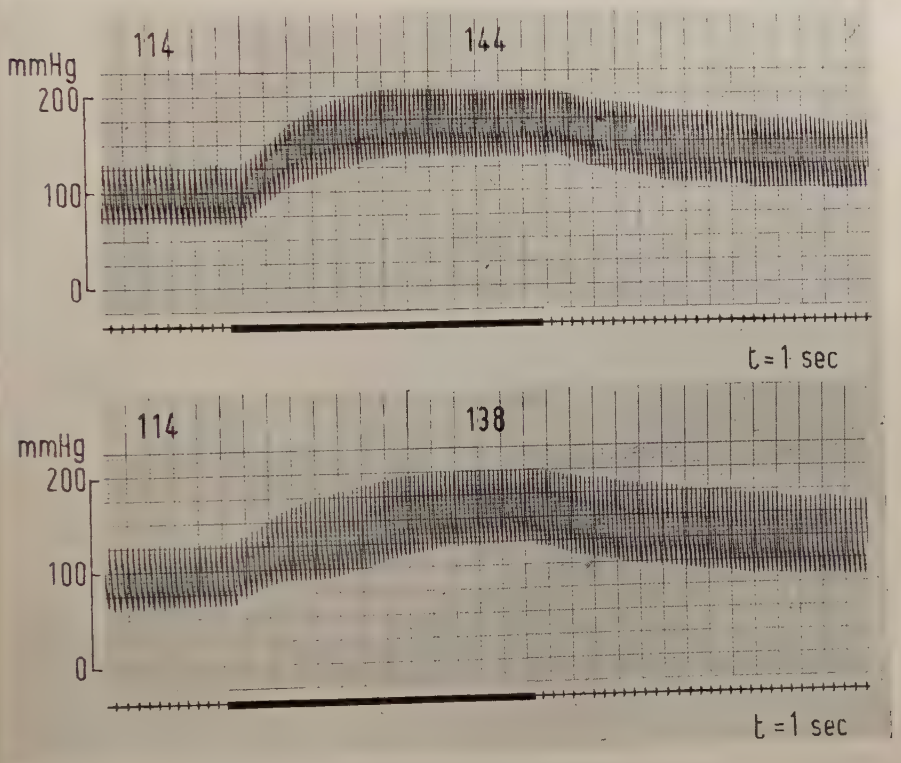

Fig. 5. – *Effects of bilateral adrenalectomy on the cardiovascular response to electrical stimulation of the posterior hypothalamus.*

Upper record: intact animal; the heart-rate, as a result of the stimulation, increases by 26.3%.

Lower record: adrenalectomised animal; the tachycardia is reduced, after the operation, to + 21%.

5. *Stimulation of the hypothalamus after removal of the stellate ganglia and bilateral adrenalectomy.* — After the combined operations in the same animal of removal of the stellate ganglia and bilateral adrenalectomy, there was a diminution in heart-rate accompanied by a slight reduction in blood-pressure. In these conditions, with the vasomotor innervation of the splanchnic region intact, stimulation of the hypothalamus still produced a hypertensive effect, although a reduced one. The heart-rate, on the other hand, remained unchanged; at the end of the stimulation, however, one could see a transitory reduction of about 8-10 beats per min (Fig. 6).

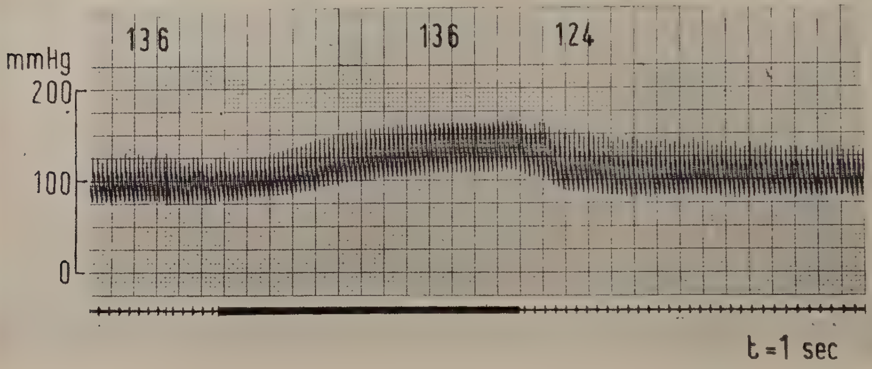

Fig. 6. – *Effects of the bilateral removal of stellate ganglia and adrenal glands on the cardiovascular response to electrical stimulation of the posterior hypothalamus.*

Same cat as in Fig. 4. The hypertensive response to electrical stimulation is still present, though reduced; in contrast, the heart-rate does not alter. At the end of the stimulation, however, one can see a diminution of 12 beats / min in the heart-rate.

6. *Stimulation of the hypothalamus after section of the spinal cord at a low cervical level.* — Complete interruption of the nervous pathways connecting the higher centres with the spinal sympathetic centres, carried out by spinal section at the level of $C_{7-8}$, produced a fall in pressure and in heart-rate, and completely abolished the appearance of any cardiac or circulatory effect following hypothalamic stimulation (Fig. 7).

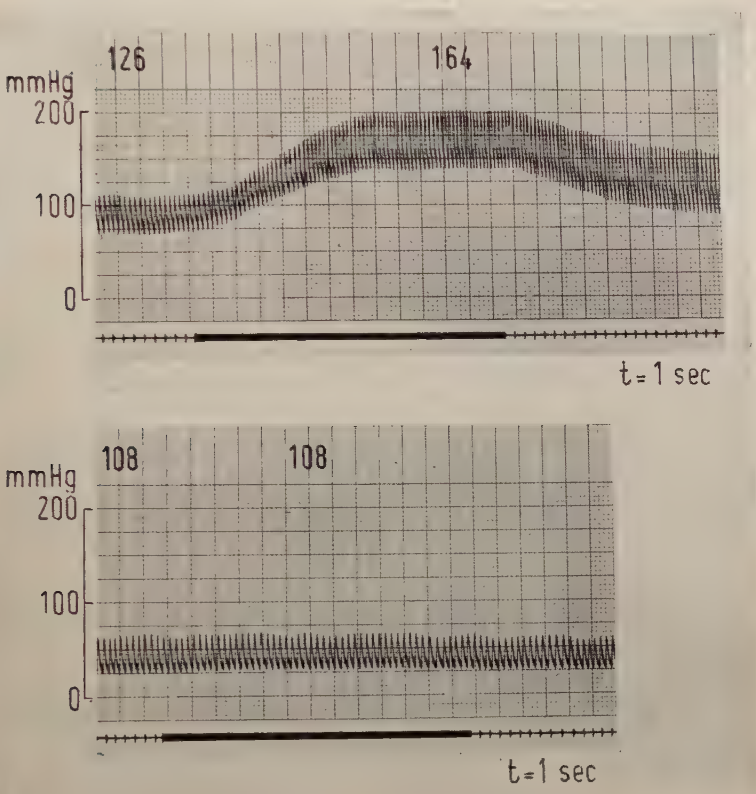

Fig. 7. – *Effects of spinal section at low cervical level on the cardiovascular response to electrical stimulation of the posterior hypothalamus.*

Upper record: intact animal; there is a marked hypertension, accompanied by a tachycardia of + 30.2%.

Lower record: after section of the spinal cord at C7-8; both the arterial pressure and the heart-rate in the control period are reduced in comparison with the previous values, and they remain unchanged by the stimulation.

7. *Stimulation of the hypothalamus after administration of hypertensive substances.* — Spinal section at low cevical level eliminated all the cardio-accelerator mechanisms and thereafter a change in heart-rate as a result of hypotalamic stimulation could come about only by inhibition of the vagal tone. If no variations in the rhythm of the heart-beats could be observed, this might mean either that the vagi were not tonically active or that the hypothalamus had no inhibitory influence on the medullary cardio-inhibitor centre. As it was likely that the vagal tone was absent as a result of the

low arterial pressure, and as it was not possible to elicit it by a hypertension from hypothalamic stimulation, all the neurohumoral mechanisms of this hypertension being eliminated, we resorted to the parenteral administration of hypertensive substances, such as the adrenal hormones or vasopressin, and we studied the changes in heart-rate which occurred during hypothalamic stimulation carried out while the arterial pressure was thus raised.

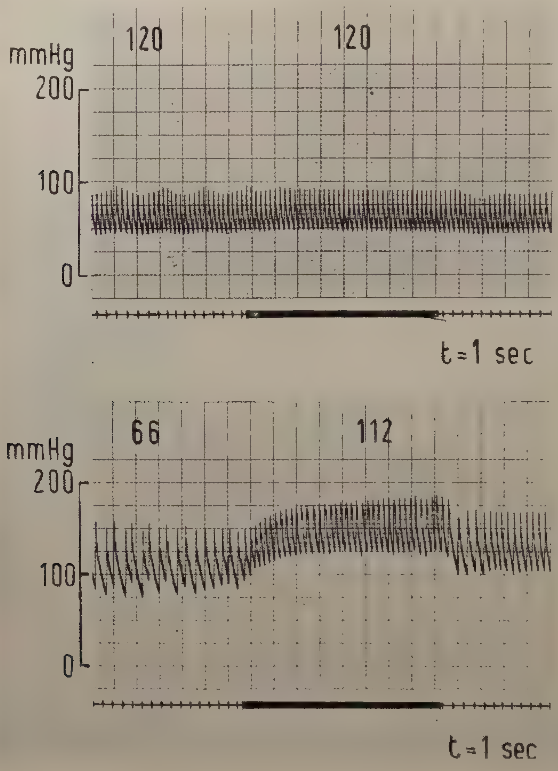

Fig. 8. – *Inhibition of the reflex bradycardiac response to nor-adrenaline, elicited by electrical stimulation of the posterior hypothalamus, in an animal with spinal section at a low cervical level.*

Upper record: animal with spinal section at $C_{7-8}$; the hypothalamic stimulation leaves both the arterial pressure and the heart-rate unaltered.

Lower record: after intravenous administration of 15 $\mu$g of nor-adrenaline, there arises a hypertension accompanied by a marked reflex bradycardia.

Stimulation of the hypothalamus blocks the bradycardiac response and causes the heart-rate to increase by 70 % over the values preceding the start of the stimulation.

In such experimental conditions the raised pressure level must necessarily have activated the aortic and carotid-sinus baroreceptors and produced reflex bradycardia. Any variation in heart-rate which appeared as a result of stimulation of the posterior hypothalamus could only have been mediated by the vagus nerves.

Adrenaline and nor-adrenaline were injected intravenously either by means of a drop-by-drop infusion at a rate of 1.2 μg/kg/min, or in a single dose of 10-15 μg. In most of the animals, but not in all, the resulting hypertension was accompanied by a reduction in heart-rate.

When a bradycardiac response did appear, it was immediately blocked by stimulation of the hypothalamus. The heart-rate increased, to fall again at the end of the stimulation. Fig. 8 shows an exemple of this. It relates to a cat in which the peripheral vasomotor mechanisms, the suprarenals and the sympathetic innervation of the heart had been excluded from the influence of the higher centres by section of the spinal cord at low cervical level; administration of nor-adrenaline had produced a marked hypertension, an intense bradycardia, and an increase in the pulse-pressure by the well-known inotropic action of this substance. In these conditions, stimulation of the posterior hypothalamus blocks the bradycardia and raises the systolic pressure still further without influencing the pulse-pressure which, if anything, tends to diminish. At the end of the stimulation, the heart-rate returns to the previous value. On the other hand, if the bradycardiac response to the nor-adrenaline passed off while the hypertensive effect still continued, hypothalamic stimulation was now completely ineffective.

An analogous sequence of effects was observed in those animals whose adrenals and stellate ganglia had been removed.

## DISCUSSION

Summarising the main points which have emerged during the experiments, one may conclude:

1) The systemic arterial hypertension evoked by electrical stimulation of the postero-lateral areas of the hypothalamus in the cat is regularly accompanied by a significant increase in heart-rate, even if the basic rate is already rather raised. At the end of the

electrical stimulation there appears, in the majority of cases, a bradycardia.

2) The tachycardiac response is not abolised, but only diminished, by section of the spinal cord at $T_{4-5}$, by removal of the stellate ganglia, or by adrenalectomy. No change in the frequency of the beats occurs, on the other hand, if the stimulation is performed after spinal section at the level of $C_{7-8}$, or after the combined operation of removal of the stellate ganglia together with adrenalectomy.

3) The bradycardia wich appears at the end of the stimulation is not abolished by removal of the stellate ganglia. One can still see a reduction in the number of beats per minute at the end of the application of the electrical stimuli, in those animals with stellate ganglia and adrenals removed in which hypothalamic stimulation no longer produces an increase in heart-rate, though it still has a hypertensive effect.

4) The bradycardia which follows administration of hypertensive drugs is blocked by diencephalic stimulation; this is followed by an increase in heart-rate, even when all the cardio-accelerator influences, nervous and humoral, have been eliminated.

In formulating the experimental problem, the question facing us was why a state of hypertension so impressive and developing so rapidly as the pressure rise from electrical stimulation of the posterior hypothalamus should always be accompanied by tachycardia, while it is well known that other states of hypertension, of nervous or pharmacological origin, produce instead a bradycardia by the cardio-aortic and carotid sinus baroreceptor mechanisms. As a hypothesis we supposed that the hypothalamus, activated by the electrical stimulation, not only excites the lower cardio-accelerator centres, but at the same time inhibits the medullary cardio-inhibitor centre.

It was necessary to show that the hypothalamus activated in this way, could actually produce changes in the heart-rate even after the elimination of all the possible cardio-accelerator influences.

We effected such an elimination by two different procedures: *a*) spinal section at a low cervical level, which interrupts all the connections between the higher centres and the sympathetic cardiac and spinal vasomotor centres; *b*) removal of the adrenal glands, together with removal of the stellate ganglia. It has been shown by various investigations (14, 17, 20) and confirmed by our experiments, that the latter procedure corresponds to a complete sympathetic denervation of the heart.

In both these conditions, the only nervous pathway to the sino-atrial node remaining intact was the vagal pathway; any variation in heart-rate, observed during the hypothalamic stimulation, must necessarily have been mediated by the vagus nerves; this also implies that the medullary cardio-inhibitory centre was tonically active.

It turned out that, after the procedures mentioned above, stimulation of the hypothalamus was completely without effect on the rhythm of the heart-beats. The difference between the two experimental conditions consisted in the fact that, after spinal section at the cervical level, it was no longer possible to elicit any hypertensive response, while in the animals with the stellate ganglia and the adrenals removed, a pressure rise was made possible by the integrity of the vasomotor innervation particularly of the splanchnic area.

The fact that a tachycardiac response was not observed in these conditions suggested an absence of vagal tone. And in fact it was established that vagotomy, carried out in these cases at the end of the experiment, left the heart-rate unchanged. It was therefore necessary to produce artificially an increase in the tone of the medullary cardio-inhibitor centre and to test in these experimental conditions the influence exerted by the hypothalamus on the chronotropism of the heart. These conditions were obtained reflexly by the administration of hypertensive substances such as the adrenal hormones; these produced a rise in pressure accompanied, if not in all, at least in a good many of the experiments, by a marked sinus bradycardia. Stimulation of the posterior hypothalamus, carried out during these conditions of raised vagal tone, caused the heart-rate to rise, cancelling completely or partially the bradycardiac effect of the injected catecholamine. Stimulation, still during the hypertensive state, was found quite ineffective when applied after the bradycardiac response had passed off.

It was therefore beyond doubt that hypothalamic activation could produce inhibition of the vagal tone, and thus a tachycardiac response, when all other accelerator influences on the heart had been eliminated.

Particular attention should be paid to the results obtained by stimulation of the postero-lateral areas of the hypothalamus after removal, in the same animal, of the adrenals and of the stellate ganglia. The absence of vagal tone in the resting condition, in spite of the good conditions of pressure, explains the absence of a tachycardiac response to hypothalamic stimulation. All the same, the increase in arterial pressure produced by the activation of the splanchnic vasomotor mechanisms by the diencephalon must have activated the mechanisms which reflexly excite the cardiac vagus, and one would then have expected, in the absence of all the cardio-accelerator mechanisms, that a reflex bradycardia would appear. The fact that no slowing of the beats is observed during the period of stimulation, can be explained by the inhibitory influence exerted by the hypothalamus on the cardio-inhibitor centre. That this is true is borne out by the fact that, on interrupting the stimulation, when this inhibition stopped while the arterial pressure was still raised, a clear reduction in heart-rate was observed.

Consequently the inhibitory action exerted by the hypothalamus on the vagal centre accounts for the post-stimulatory bradycardia which occurs in the experimental conditions described above without any previous tachycardiac response.

A similar interpretation could apply to the reduction in heart-rate which occurs in the intact animal at the end of the stimulation, preceded in this case by a tachycardia. During the diencephalic activation, impulses of baroreceptive origin are converging on the medullary centre of the vagus, but this is under the influence of the inhibition from the hypothalamus which, moreover, is at the same time activating the cardio-accelerator mechanisms. It is only when the inhibition also is eliminated, at the end of the sympathetic stimulation, that the signs will appear of an increase in vagal tone in response to the raised arterial pressure. This is because the hypertension, depending on initiating mechanisms of considerable inertia, continues for a certain time after the end of the stimulation and only dies away slowly, in contrast to the nervous phenomena which produced it, which stop at the end of the application of the electrical stimuli.

The post-stimulatory bradycardia, in the intact animal, could in fact also be produced by the cessation of the tonic cardio-accelerator impulses at the end of the diencephalic stimulation, since it has been shown that at the end of the stimulation there follows an immediate oscillographic silence in the fibres of the cardiac nerves, indicating an inhibition of the normal tonic activity of these nerves (16). This mechanism cannot be held to be the only factor if one takes into account the fact that the bradycardiac response referred to is not abolished by bilateral removal of the stellate ganglia.

On the basis of the experimental results discussed above, it seems possible to conclude that playing a part in the origin of the hypothalamic tachycardia there are not only sympathetic and hormonal activating mechanisms but also a mechanism of inhibition of the parasympathetic centres.

Such a hypothesis has never been taken into account by the authors who have studied this topic, although it is based on the principle of reciprocal innervation in the autonomic system. Wang and Ranson (21), who have made a particular study of the role of the hypothalamus, both anterior and posterior, in the regulation of the heart-rate, and who have carried out some experiments similar to ours, make no mention of a possible inhibitory influence exerted by the posterior hypothalamus on the medullary centre of the vagus; while, on the other hand, they state that the bradycardia of preoptic stimulation is only partly an effect of excitation of the vagus, and postulate an inhibition of the sympathetic cardio-accelerator centres. It is surprising that a similar hypothesis for the cardiac effects of hypothalamic stimulation has never been put forward, while a two-fold mechanism has been shown for the pupillary dilatation of similar origin: one by sympathetic excitation of the dilator muscle of the iris, and one, more important than the preceding one, at least in the cat, by inhibition of the parasympathetic centres which control the constrictor muscle of the pupil (10, 12, 13, 22).

The only evidence that can provide indirect support for a two-fold hypothalamic influence, excitatory and inhibitory, on the heart-rate, comes from the investigations of Bond (3) on sympathetic and vagal interactions in the responses of the heart to emotional stimuli. He subjected dogs and cats to emotional stimuli and found that the observed changes in heart-rate are due not only to an activation of the sympathetic system, but also to a simultaneous inhibition of the vagal tone. Nevertheless, even if this could be considered a proof, albeit indirect, of what has been postulated above, we still do not know what are the reciprocal parts in the cardiac response to the emotional stimuli which are played by the posterior sympathetic areas and by the anterior parasympathetic areas of the hypothalamus.

Another interpretation of the hypothalamic tachycardia calls for examination. That is, one might suppose that, in the intact animal, the activation of the cardio-accelerator nerves would be such as to produce an increase in heart-rate, counterbalancing and overcoming any reflex inhibitory influence on the sinus rhythm. One would be dealing, on such a hypothesis, with the algebraic sum of two effects, one of which was more potent than the other. The nervous impulses starting from the cardio-inhibitor centre when it is activated reflexly by the pressure-rise, and travelling along the trunk of the vagus, would arrive at the specialized tissue of the sino-atrial node when this is already under the influence of a very considerable and preponderant activation from the cardio-accelerator nerves. But the tachycardia of hypothalamic stimulation remains even after removal of the two stellate ganglia. In this case it is undoubtedly produced by the adrenaline or nor-adrenaline liberated by the adrenal glands. But it is known, and indeed has been shown in our experiments, that when these substances are injected into experimental animals with vagal and depressor nerves intact, they usually produce a diminution in the heart-rate. On the other hand, no bradycardiac response was observed at the beginning of the hypertensive state in cats whose stellate ganglia had been removed.

Lastly, we should consider the interpretation recently given by Gellhorn (5) of the bradycardia which appears at the end of a period of stimulation of the posterior hypothalamus. He interprets this as a manifestation of an increase in parasympathetic activity occurring at the end of the sympathetic effect. This phenomenon, analogous to Sherringtonian successive induction, would take place exclusively at hypothalamic level, in accordance with the reciprocal functional interconnections, which are held by this author, on the basis of earlier work, to exist between the sympathetic areas, posteriorly, and the parasympathetic areas, anteriorly, of the base of the diencephalon (cfr. 4). However, it is not clear how, in this case, an effect of autonomic reciprocal innervation could be present only at hypothalamic level and not at the level of the medulla, when in other experimental conditions the effect does occur at medullary level. In the second place, if an increase in cardio-inhibitor tone arises reflexly through the baroreceptor system, it will show itself principally at the level of the medulla, this being the place where the afferent pathways from the presso-receptors of the vasosensory regions especially converge and also the place where the nucleus of origin of the cardiac vagus is situated. When one considers also the fact that at least a good number of the pathways which descend from the posterior hypothalamus to influence the lower autonomic centres, converge on the centres of the medulla oblongata, it becomes clear that an interpretation which takes account of the experimental findings already available and of the more recent evidence must be one of interaction at the level of the medulla. This does not exclude the possibility that the principle of reciprocal innervation may be valid also for autonomic levels above the medulla.

## SUMMARY

In experiments on electrical stimulation of the postero-lateral areas of the hypothalamus in the cat, it has been found that:

1) Hypothalamic stimulation produces, together with a marked hypertension, an increase in heart-rate of 20-30% and more. In most cases the cessation of the electrical stimulation is followed by a bradycardia.

2) Elimination of the adrenal glands alone, by bilateral adrenalectomy, or of these and at the same time of the vasomotor supply to the splanchnic area, by section of the spinal cord at $T_{4-5}$, does not produce any appreciable change in heart-rate. The hypertension and tachycardia on hypothalamic stimulation can have an exactly comparable intensity to that obtained in the intact animal, but in most cases they are slightly reduced in amount.

3) Elimination of the sympathetic innervation of the heart by removal of the two stellate ganglia leaves the cardio-circulatory conditions unchanged and does not prevent the development of hypertension and tachycardia. The rise in pressure is equal in amount to that in the control experiments but it begins with a delay of some seconds from the onset of the stimulation; the tachycardia is quantitatively lessened.

4) After elimination of the adrenal glands and stellate ganglia, stimulation of the hypothalamus still produces a rise in arterial pressure, but leaves the heart-rate unchanged.

5) Spinal section carried out at the level of $C_{7-8}$ makes the hypothalamic stimulation quite ineffective.

6) The bradycardia which appears at the end of the stimulation is not abolished by removal of the stellate ganglia. A reduction

in the number of beats per minute can still be observed, at the end of the application of the electrical stimuli, in those animals in which, by removal of the adrenals and stellate ganglia, hypothalamic stimulation no longer produces changes in heart-rate, while still producing a certain hypertensive effect.

7) The bradycardia which follows administration of hypertensive drugs, such as adrenaline, nor-adrenaline and vasopressin, is blocked by stimulation of the hypothalamus. Such stimulation is followed by an increase in heart-rate, not only in the intact animal, but also after section of the spinal cord at a low cervical level, which eliminates all the nervous and hormonal cardio-accelerator influences.

The results suggest that in the genesis of the tachycardia by stimulation of the postero-lateral areas of the hypothalamus, there come in as factors not only mechanisms of sympathetic and adrenal activity, but also a mechanism of inhibition of the medullary cardio-inhibitor centre.

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# BINOCULAR FACILITATION IN THE LATERAL GENICULATE BODY

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## INTRODUCTION

The experiments to be described in this report were performed in order to investigate the possibility that the discharge of principal neurons in the dorsal nucleus of the lateral geniculate body (LGD) innervated by one of the optic nerves might be facilitated by high-frequency activity in the other optic nerve.

Prominent after-positivity in the LGD follows each stimulus to one of the optic nerves at repetition rates less than 10/sec (11) and is accompanied by marked depression of the post-synaptic spike response to stimulation of the other optic nerve (15). This after-positivity has been attributed to the hyper-polarizing synaptic action upon principal cells of inhibitory short axon cells innervated by recurrent collaterals from the axons of principal cells (15). At repetition rates greater than about 10/sec the conditioning stimuli no longer elicit after-positivity (14) and depression of the post-synaptic spike response to test stimulation of the other optic nerve disappears (16). At repetition rates greater than 25/sec a DC response may be recorded in the LGD which has been attributed to maintained depolarization of principal cells due to the summation of excitatory post-synaptic potentials elicited by repetitive optic nerve fiber activity (12). This DC response increases logarithmically with increasing stimulation frequency to a maximum amplitude at about 300/sec. It was believed that facilitation of the spike response of principal cells to stimulation of one optic nerve would be most prominent, if present at all, when conditioning stimulation of the other optic nerve was delivered at these high frequencies. Also, it seemed likely that such facilitation as might be found would exhibit a close relationship to the DC response to conditioning stimulation.

## METHODS

These observations were made in the course of 24 experiments on cats with inter-collicular transections of the brain stem. The surgery was performed several hours before recording. Surital and Trilene, alone or in combination, were used for anaesthesia in as small amounts as possible. Both optic nerves were exposed by removal of the extra-ocular muscles and orbital fatty tissue. In most experiments the globe and its blood supply were intact, but in some the anterior half of the globe was resected and the remaining portion sutured together to render the retina inoperative. In 16 of the 24 cats the LGD on the side of recording was isolated from pretectal and medial thalamic structures by a parasaggital section which passed by its medial edge along its entire antero-posterior length. This section was made with a long, thin knife which was inserted into the rostral portion of the mid-brain after retraction of the posterior portion of the cerebral hemisphere and rotated in a dorso-ventral direction. In 5 of these 16 cats the LGD on the other side was either similarly isolated or entirely removed by aspiration. All brains were examined in 1 mm sections after fixation in formalin.

Rectangular pulses of 0.05 to 0.1 msec duration were delivered to each optic nerve by 2 silver electrodes, one thrust into the center of the nerve and the other encircling it just behind the globe. Stimuli of about two thirds the intensity for maximal response were used for both conditioning and testing stimuli. Responses were recorded in the LGD with a double-barrelled glass capillary electrode with one barrel broken away from the tip for a distance of 1.5 mm Outside diameter at the tip was about 100 $\mu$. This electrode was filled with 3M NaCl and connected by saline bridges and calomel half-cells to a DC amplifier. The LGD was probed in a vertical direction through intact cortex, and the elctrode was placed for maximum amplitude of the DC response, a point usually reached in previous experiments (11, 13) when the lower tip reached the A layer (terminology of Thuma, 10). Responses were recorded in the visual cortex with a bipolar electrode made of 2 insulated Nichrome wires 50 $\mu$ in diameter cemented together with a tip separation of 1 mm. This electrode was placed vertically in the cortex so that the lower tip lay in its middle third. Connection was made to the amplifier input by fine wires which permitted the electrode to move freely during respiratory and other movements. It may be noted that both the geniculate and cortical electrodes were of such size as to record only the synchronous discharge of many neuronal elements, and expressions such as " radiation spike " which will be used in this report should, therefore, be understood to refer to population responses and not to the activity of single units.

In order to minimize spike components in the geniculate response to repetitive stimulation, the amplifier used for DC responses in the geniculate was set for a low band pass such that a 100 c/s signal was down 1/2 amplitude by placing an R-C leak to ground from the control grid in the last stage of amplification. It was ascertained that no DC response was recorded under these conditions when repetitive rectangular pulses similar in strength, duration and frequency to those spike components of the geniculate response were led directly into the amplifier. No DC response to repetitive optic nerve stimulation is recorded under similar experimental conditions after death of the animal or after the optic nerve is crushed proximal to the stimulating electrodes (11). In order to minimize all components of the cortical response except the rapid radiation spike, the amplifier was operated with a short time constant (0.001 sec) when recording on a slow time line as in the experiments of Figs. 3 and 4.

Responses were displayed on a CRO and photographed in the usual manner. Negativity at the lower electrode tip is signalled by a downward deflection in the geniculate records of the DC response and by an upward deflection in the transients recorded in the visual cortex. Some of the fast portions of the responses in Figs. 1 and 4, A were retouched for better reproduction.

## RESULTS

The post-synaptic response of principal neurons to stimulation of the contralateral optic nerve at 0.8/sec recorded in the LGD was found to be little altered during repetitive stimulation of the ipsilateral optic nerve at rates between 35/sec and 300/sec. When recorded in the cortex as the radiation spike this response was occasionally found to be slightly increased in amplitude when the average of a large number of responses was considered. Marked increase in amplitude of the radiation spike responses was consistently found, however, when they were elicited at frequencies greater than 10/sec. Fig. 1 shows the results of a typical experiment. The radiation spike is identified as the first deflection. The onset of this up-going deflection may be attributed to the arrival of the propagated action potential in radiation fiber terminals in the middle layers of the visual cortex (1). Its latency varied from one experiment to another between 1.5 and 2.2 msec and in this experiment was 1.5 msec. Average radiation spike amplitudes in this experiment were 175,44 and 98 $\mu$V for the unconditioned test responses at 0.8/sec, unconditioned test responses at 25/sec, and conditioned test responses at 25/sec respectively. The gradual decline in test spike amplitude to control values following the end of conditioning stimulation was the usual finding. Since the amplitude of the radiation spikes in these experiments may be accepted as a measure of the number of active radiation fibers, the observed increases in amplitude may be interpreted as an increase in the number of principal neurons from which each presynaptic test stimulus elicited a propagated action potential. It seems proper, therefore, to use the word "facilitation" in the further description of these observations. In the experiment of Fig. 2 test stimulation of the contralateral optic nerve was delivered at 70/sec and the radiation spike amplitudes of the unconditioned responses at 0.8/sec, unconditioned responses at 70/sec. and condi-

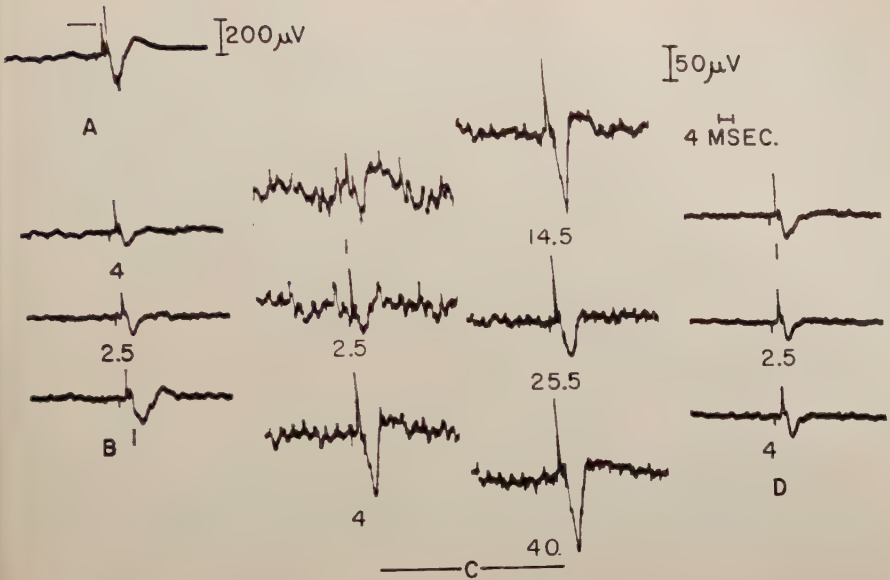

Fig. 1. – *Facilitation of optic radiation spike responses to stimulation of contralateral optic nerve at 25/sec during conditioning stimulation of ipsilateral optic nerve at 300/sec.*

*A*: response to test stimulus at 0.8/sec, peak of radiation spike indicated by marker. *B*: representative test responses at 25/sec. 4, 2.5 and 1 sec before onset of conditioning stimulation. *C*: representative test responses at 25/sec. 1, 2.5, 4, 14.5, 25.5 and 40 sec after onset of conditioning stimulation. *D*: representative test responses at 25/sec. 1, 2.5 and 4 sec after end of conditioning stimulation. Note increased gain in *B*, *C* and *D* and decreased amplitude of radiation spike in *B* compared with *A*.

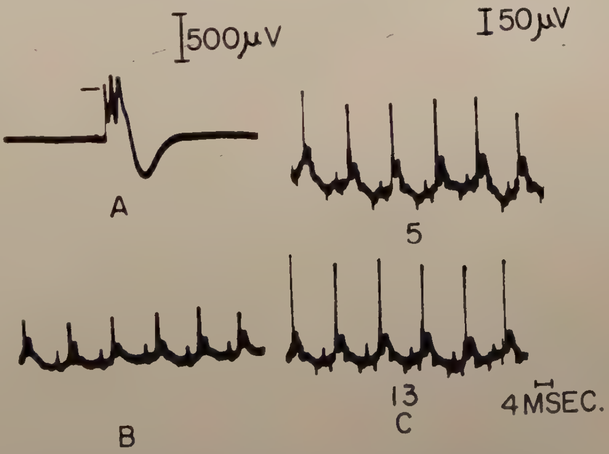

Fig. 2. – *Facilitation of optic radiation spike responses to stimulation of contralateral optic nerve at 70/sec during conditioning stimulation of ipsilateral optic nerve at 250/sec.*

*A*: representative test response at 0.8/sec. *B*: representative test responses at 70/sec before conditioning stimulation. *C*: representative test responses at 70/sec. 5 and 13 sec after onest of conditioning stimulation. Note increase in gain in *B* and *C* and decreased amplitude of radiation spike in *B* compared with *A*.

tioned responses at 70/sec were 600, 100 and 215 μV respectively. The test responses to repetitive stimulation in the experiments of Figs. 1 and 2 were increased by 122 and 115% respectively. Facilitation varied from experiment to experiment between 30% and 200%. In no instance was it of sufficient magnitude to restore the spike amplitude to its maximum value as found by stimulation at 0.8/sec. The degree of facilitation in any given experiment was found to increase with increasing frequency of conditioning stimulation between 50/sec and 300/sec. Changes in facilitation over the higher range of conditioning frequencies, however, were small, and extensive records were difficult to obtain over the lower range because of intermingling in the cortical record of conditioning and test responses. At frequencies greater than about 100/sec the radiation spike responses to conditioning stimulation usually disappeared from the cortical record, affording thus a clear view of the responses to test stimulation.

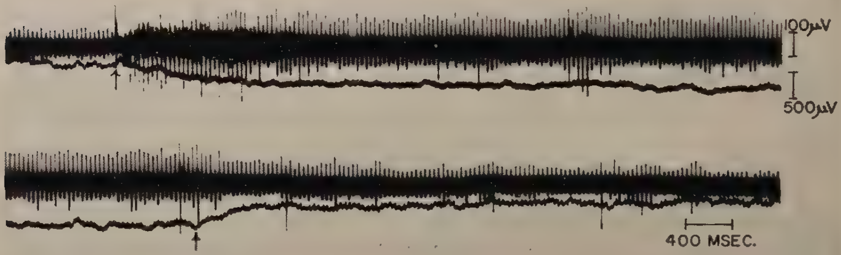

Fig. 3. – *Radiation spike responses (upper line) to contralateral optic nerve stimulation at 24/sec during DC responses in LGD (lower line) to ipsilateral stimulation at 300/sec.*

Onset and end of conditioning stimulation marked by arrows. Record continuous.

The DC response in the LGD to high-frequency conditioning stimulation may be recorded simultaneously with the radiation spike responses to test stimulation as in Fig. 3. The appearance of radiation spike responses to conditioning stimuli in the first few seconds of stimulation and depression of the test radiation spike responses during the same period due to after-positivity in the LGD elicited at the onset of conditioning stimulation (13) made it difficult to find a clear relationship between development of facilitation and build-up of the DC response. The persistence of both phenomena for periods of many seconds of conditioning stimulation was seen, however, in all experiments, and the degree of facilitation was found to increase with increasing amplitude of the DC response as produced by increasing the conditioning stimulus strength or frequency. The decay of both states at the end of conditioning stimulation always occurred along time courses so similar as to suggest a closer relationship than one of simple coincidence. Fig. 4 shows the decay of facilitation following the end of a 10 sec period of conditioning stimulation in two other representative experiments. In experiments of this kind it was usually necessary to examine the responses recorded by the cortical electrode on a fast time line in order to be sure that the spikes on the slow time line were really radiation spikes and not those portions of the response resulting from activity in cortical elements. These latter portions of the response are generally small under conditions of repetitive optic nerve stimulation and were further minimized by proper location of the electrode and by use of a short time constant.

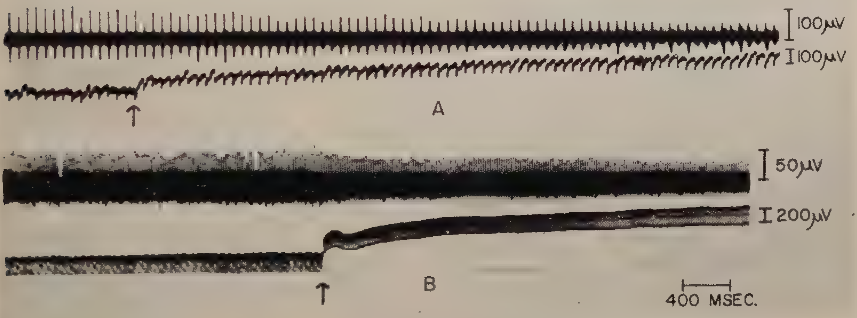

Fig. 4. – *Records from two experiments showings similarity in time course between declining facilitation of optic radiation spike responses (upper line) to stimulation of contralateral optic nerve and declining DC response in LGD (lower line) to ipsilateral stimulation at end of ipsilateral stimulation (marked by arrow).*

Contralateral stimulation 14/sec in *A* and 43/sec in *B*; ipsilateral stimulation 300/sec in *A* and 250/sec in *B*.

Examination of the fixed brain sections revealed that in most experiments isolation of the LGD from pre-tectal and medial thalamic structures was complete or nearly so, and there were no differences in experimental results which could be attributed to differences in the degree of isolation. The section in Fig. 5 from the experiment of Figs. 1 and 3 are typical of those in which isolation was complete. Observations in intact preparations were the same as in those with isolation of the LGD.

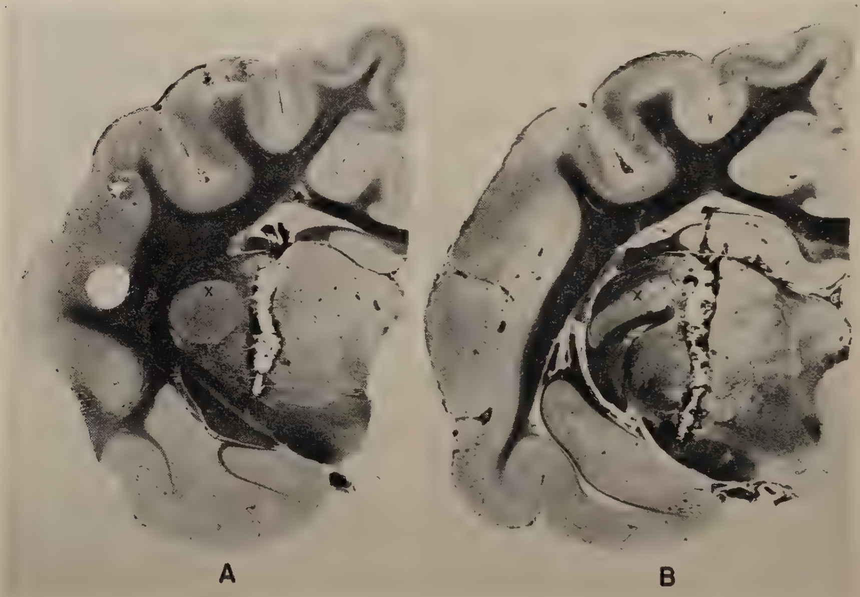

Fig. 5. – *Coronal sections through anterior* (A) *and posterior* (B) *portions of LGD* (*marked by X*) *in experiments of Figs. 1 and 3.*

Note complete isolation of LGD and optic tract from all medial structures. Myelin stained by modified Pal-Weigert method. Magnification 5.7 X.

Observations of the DC response and of facilitation in preparations with globe resections were the same as in those with intact retinas.

It was usually found more satisfactory to apply test stimuli to the contralateral optic nerve because of the larger size of the radiation spike, but the same observations as in Fig. 1-4 were made when test responses to ipsilateral stimulation were conditioned by high-frequency stimulation of the contralateral nerve.

## DISCUSSION

Depression of the radiation spike response at stimulation frequencies greater than 1/sec (Fig. 1 *B*; Fig. 2 *B*) represents the failure of some principal neurons to develop a propagated action potential in response to each optic nerve stimulus. This failure, whatever may be its mechanism, suggests the existence of a pool of neurons in the state of sub-liminal excitation, and the facilitation described in this report would appear to confirm this suggestion.

Anatomical isolation of the LGD from medial thalamic and pretectal structures has limited the facilitation described in these experiments to processes located entirely within the specific visual system. It may be of interest to note that repetitive optic nerve stimulation under these experimental conditions has not been found to alter the spindling pattern of spontaneous cortical activity as recorded by the EEG (unpublished observations).

Similarity in time course suggests that facilitation of responses to test stimulation and the DC response to conditioning stimulation have a causal relationship. On the basis of its spatial distribution (11) and because of its close relationship to facilitation of the geniculate spike response to antidromic stimulation (13), it has been inferred that the DC response to repetitive optic nerve stimulation represents maintaned depolarization of the cell bodies, and possibly dendrites, of principal neurons. It seems probable that this depolarization is maintained by the summation of a series of excitatory post-synaptic potentials, an event which has been observed with micro-electrodes during repetitive presynaptic stimulation of anterior horn cells (2, Fig. 43) and giant motor axons of the crayfish (3). It may be suggested, therefore, that the discharge of principal neurons innervated by fibers in the optic nerve used for test stimulation is facilitated by the steady depolarization of these cells due to repetitive synaptic stimulation by fibers in the nerve used for conditioning stimulation. The observation reported elsewhere (15) that the amplitude of the radiation spike response to simultaneous optic nerve stimulation is simply the sum of the values obtained by separate stimulation may be interpreted in the light of this hypothesis by assuming that innervation of principal neurons receiving optic nerve fibers from both eyes is asymmetrical to such a degree that under the conditions of single shock stimulation a propagated action potential is elicited by activity in fibers from only one of the optic nerves. In this case, activity in fibers from the nerve of weaker innervation is presumed to have only a facilitatory action.

The degree of facilitation observed in these experiments would seem to require a larger number of principal neurons with innervation by fibers in both optic nerves than is permitted by the histological findings of Hayhow (4), who has studied the distribution of optic nerve terminals in the LGD by the method of Nauta. The regions of pre-terminal degeneration shown by this author to be common to both optic nerves do not seem to compose more than 5% of the total LGD volume [1], whereas in the experiments of Figs. 1 and 2, for example, the radiation spikes showed an increase in amplitude during conditioning stimulation of 54 and 115 microvolts respectively, 30% and 19% of the amplitude of the responses to unconditioned test stimulation at 0.8/sec. It must be recognized, however, that this discrepancy between histological and electrical findings may be more apparent than real for several reasons among which may be mentioned: *a*) ignorance of the form of the function relating radiation spike amplitude to number of participating elements; *b*) ignorance of the relative contribution from cells in the region of over-lap to any given test response. At the present time this explanation for facilitation seems the most probable one, particularly when the alternatives discussed below are considered.

An alternative mechanism for facilitation might be based upon the possible effects of extra-cellular current flow upon the thresholds of cell bodies and axons in its field. The DC response to repetitive stimulation is of the same order of magnitude as the transient post-synaptic response to a single stimulus and has been shown to have the same spatial distribution as the post-synaptic transient when recorded with macro-electrodes of the same size as those used in the experiments of the present report (10). It is, therefore, reasonable to consider that the DC extra-cellular current flow has approximately the same strength and distribution about a single generator unit as does the transient current flow associated with the spike discharge of a single unit. No observations of the latter are available for the LGD, but Mountcastle, Davies and Berman (5) have described the spike potential field about a single unit in the sensory cortex in which the maximum gradient was found to be 200 $\mu$V/20 $\mu$ or 0,01 V/mm (calculated from points in graph of Fig. 7 in ref. 5). Assuming an average specific resistance for grey matter of 500 $\Omega - \text{cm}$ (8, p. 60; 9, p. 470) or 5000 $\Omega$ for a cube 1 mm along each edge, the total current flow across one face of this cube normal to its surface and in the direction of the potential gradient of 0.01 V/mm is $0.01/5000 = 2 \cdot 10^{-6}$ amp. Strumwasser and Rosenthal (7) have reported their success in altering the firing pattern of single units in the cortex of frog brains by currents of $0.5\text{-}10 \cdot 10^{-9}$ A delivered through micro-electrodes of less than 0.5 $\mu$ diameter. The current density at the electrode orifice in these experiments may, therefore, be calculated to have been $0.4 - 9 \cdot 10^{-2}$ A/mm. This value is greater than that estimated for the extra-cellular current generated by a single unit by a factor of $10^4$. The magnitude of this difference suggests that extracellular current flow does not affect the threshold of neurons in its field, even when the most favorable conditions for its action are assumed, and the facilitation observed in our experiments would seem to require a purely synaptic mechanism.

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[1] Bishop, P. O. *et al.* (*Science, 130*: 506-507, 1959) have found post-synaptic units in the LGD which discharged following stimulation of either optic nerve, but the number was small (23 of 270 units studied) and the identification of most of them as principal neurons uncertain.

A third mechanism for facilitation which should be considered depends upon the possibility that principal neurons discharged by the conditioning stimuli activate by recurrent collaterals interneurons in the LGD which in their turn elicit excitatory post-synaptic potentials from principal neurons innervated by the test stimuli. Short axon cells in the LGD which apparently terminate upon principal neurons have been described by O' Leary (6), and their operation in an inhibitory pathway has already been inferred in order to explain binocular inhibition in the LGD (15). In the event that some of these short axon cells have a facilitory function as well, part of the DC response would be presumed to represent summated post-synaptic potentials elicited from principal neurons by repetitive activity in excitatory interneurons. Strong evidence against this view is provided by the finding reported elsewhere (12) that repetitive antidromic stimulation of the LGD does not elicit a DC response from principal neurons and is not associated with facilitation of responses to orthodromic or antidromic stimuli.

## SUMMARY

1. The amplitude of optic radiation spike responses to repetitive stimulation of one optic nerve has been shown to be considerably increased in amplitude during continuous high-frequency stimulation of the other optic nerve. This phenomenon has been interpreted as the result of a facilitatory action upon the development of propagated action potentials in principal cells of the LGD.

2. Facilitation has been shown to be closely related in time course and amplitude to the DC response in the LGD to the conditioning stimulation.

3. It is suggested that the most probable mechanism for facilitation has its anatomical basis in the overlapping innervation of principal neurons by fibers from both optic nerves. It is also suggested that facilitation results from maintained depolarization of principal neurons by repetitive pre-synaptic activity in fibers in the optic nerve selected for conditioning stimulation and that the DC response is an electrical sign of this facilitation.

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# ANALYSES

COMMUNICATIONS Biophysics Group of Research Laboratory of Electronics and William M. Siebert, *Processing neuroelectric data*. The Technology Press of Massachusetts Institute of Technology. Cambridge (Mass.), 1959, VII-121 pp., $ 4.00.

The aim of this short monograph is to give a quick and concise account of the achievements of some newly developed methods for processing data from electrical activity of the nervous system.

The basic ideas on which the Communications Biophysics Group has worked in the last few years are laid down in the first chapter by Dr. W. A. Rosenblith: " The quantification of neuroelectric activity ". Attempts to describe neural activities quantitatively are not new and have been applied with success to the physiology of nerve fibres. However, it is a rather general opinion amongst neurophysiologists that activity of complex nervous aggregates such as the brain has escaped so far all attempts of quantification. The enormous number of neurons in the brain and the intricacy of their interactions are a measure of the magnitude of the task. Physicists as well as neurophysiologists have attempted to describe the behavior of complex neural networks by constructing electronic models of neurons and their connections. A considerable degree of complication of interactions has already been achieved, but these efforts are still very far from the actual complexity found in the living brain. Difficulties arise mainly from the fact that an increase in the number of " neurones " in the model brings about a geometrical increase of the network to account for all possible interactions. The model, therefore, gains quickly in bulk and becomes impractical in a short time.

Without completely abandoning this latter aspect of the problem, the techniques described in this monograph are devoted to the quantitative treatment of activities recorded by gross electrodes, in which all-or-none events are inextricably mingled with graded phenomena. EEG analysers developed mainly for clinical work are representative instruments designed for this purpose. Since records often contain a much larger amount of information than is detected by visual inspection, the displays from these devices generally supplement the EEG recordings and thus tend to increase the amount of information displayed, with the hope of catching meaningful details sufficient to describe the behavior of the whole in special situations.

The central question that the Biophysics Communications Group asks is (p. 2) " ... can we find selective transformations upon the data that yield meaningful description while reducing the total amount of information displayed ? ".

It is worthwhile to consider the aspects of this question. One may remark, first of all, that the attention is devoted to the processing of the data, not to the actual way of collecting them. This methodology, therefore, would be applicable by every neurophysiologist on every record of brain activity. The emphasis of the whole endeavour is put into the effort of transforming the data obtained and the novelty of the approach lies in the attempt to shrink the bulk of information to a much simpler display. This approach assumes that the raw data contain irrelevant and redundant information which may be removed or reduced in the processing. The difficulties of the task are represented by the necessity of preserving the *meaningful* aspects of the recorded activity in the transformed data.

Dr. Rosenblith's views on this problem are outlined at p. 5: "Only pluralistic strategy guarantees, at this stage of our knowledge of the nervous system, that we shall not blind ourselves to useful approaches because we have oversold ourselves on one of them". From this derives the necessity of developing not one, but several methods of quantification. As the phenomena might not be immediately "... characterized by absolute numbers, but rather by distinctive features or parametrically defined patterns..." (p. 4) a long and painstaking work is needed for *each* phenomenon in order to pin-point the distinctive marks and allow numerical description of its parameters.

The second part of Dr. Rosenblith's chapter is devoted to the statistical approach to neuroelectric phenomena. In this reviewer's opinion the description of the statistical approach deserved larger space. It is true that basic ideas on statistics are outlined in Appendix B, and the more useful applications are mentioned in chapters 2 and 3. Still, it might have been appropriate to furnish more extensive discussion of the methods for handling data obtained with macro and micro-electrodes, as well as of the applicability of the mathematical models of random processes in different instances.

Chapters 2 and 3 deal with the attempts of data quantification applied to the two main problems: the analysis of the evoked responses and the EEG processing.

"Evoked responses" (chapter 2) by M. H. Goldstein, Jr. and W. T. Peake (with C. D. Geisler, N. Y-S. Kiang, T. T. Sandel and J. S. Barlow) represents the main field of interest of the Communications Biophysics Group. The problem of data processing arises mainly because the responses evoked by repeated application of identical stimuli generally present considerable variations of amplitude, form and latency.

The chief contribution of this chapter is the description of suitable "averaging" techniques for evoked responses. The application of analog and digital computers offers a fast and reliable way of processing. Averaging appears well suited when a high degree of "background" activity conceals the evoked response to be studied. The final result of this method based on the mathematical model of random processes is that those events which are repeated at the same time in each cycle are summated while random events tend to average towards zero. The averaging process does not allow a quantitative evaluation of the share in the evoked activity of those elements which are also active independently from the stimulus. This however is a problem which deserves a further development. A beginning in this direction is represented by a method, described at the end of the chapter, of plotting the median and interquartile range of the evoked responses against time.

One is immediately aware of the advantages of working with "averaged" responses when dealing with problems such as latency, waveform etc., since the averaged response represents a more stable voltage-time envelope than any individual response.

This technique requires that during the time of averaging the whole system being studied is in a *stable steady state*. Working with threshold stimuli and long stimulus intervals one may succeed not to disrupt the stability. However, working at threshold as well as decreasing the stimulation rate, entails the need for a very large number of stimuli and long averaging time, during which the conditions may become unstable. An alternative method, when it is possible to apply it, is offered by high rate stimulation. In this case one may again obtain a steady state, although different from the initial one. The averaging of the high rate evoked responses begins only after a period of transition when the state stabilizes at the new level.

Anaesthesia deeply modifies also the general conditions of the system studied, but has the advantage of increasing stability. Examples of averaging of evoked responses with and without anaesthesia are well illustrated in the monograph.

C. E. Molnar, T. F. Weiss and C. D. Geisler present in chapter 3 "Two techniques for processing EEG data". This chapter deals mostly with sorting out from long stretches of EEG recordings those characteristics which can help to define a "state".

The main development of this team is the application of the autocorrelation technique. It is also based on the mathematical model of random processes and its usefulness lies in the detection and quantitative appraisal of rhythmically recurrent voltage changes. The operations leading to this transformation are quite complex but are performed exclusively by the computer. The results seem particularly striking when dealing with records displaying a considerable and irregular background of "spontaneous" activity, or when comparing the activity of two near-by or distant areas (by cross-correlation). It is remarkable that such a technique has been applied so far only in few occasions and mostly in clinical work, whereas it seems particularly suited in the electrophysiological laboratories, applied to animal experimentation, where the changing conditions of the brain are less easily monitored. The only examples brought out in this chapter are, in effects, related to alpha activity in man.

The autocorrelation technique allows a greater resolution than usual analyses, in that a refined analysis of the frequency of the rhythmic activity is actually performed by relating the correlation coefficient, $R$, of a point in a voltage plot to the same point of the same plot but shifted in time by the interval $\tau$ (usually milliseconds). It represents, therefore, an extension of the use of correlation coefficients between activity of two adjacent points. It is reasonable to expect that this technique will yield data of great value in defining steady states. It is however only one of the several approaches that Dr. Rosenblith recommends in the introduction chapter and should be supplemented by data from more or less conventional wave analysers and from averaging of evoked activity.

In chapters 2 and 3 are also described more conventional applications of computers to statistical treating of data from evoked responses and EEG. In chapter 2 the ALMIDO (Amplitude and Latency Measuring Instrument with Digital Output) plots histograms of voltage-time relationships of evoked responses. Any characteristic feature can be tabulated but of course the selection of the features to be plotted is a more or less arbitrary choice of the experimenter. The same is true for the device described in chapter 3. Here a computer is programmed to tabulate the data related to burst activity. To the experimenter is left the choice of the criteria by which a burst is defined. In these types of processing previous knowledge of the waveform of the events of interest is of primary importance. The choice of the criteria in both cases must be a consequence of a *previous analysis* of the recordings being analysed, or of similar activity. The computer can give only the quantitative aspects of a problem already defined on qualitative grounds.

After short concluding remarks, the monograph is completed by appendices on "The description of random processes" by William M. Siebert, "Mathematical statistics" by M. H. Goldstein and T. F. Weis and finally a "Description of computers" by R. M. Brown, J. S. Barlow, D. F. O'Brien, W. T. Peake and C. D. Geisler. Appropriately, these appendices form a large portion of the book. Indeed, no complete understanding of the quantitative techniques can be obtained without a solid background of physics and mathematics.

The book is intended "…. to convince young researchers from the physical and life sciences of the desirability of acquiring the gamut of necessary skills... " (p. 64). However, being written by engineers and mathematicians, it is addressed mainly to biologists who, as a rule, do not possess sufficient knowledge of the mathematical background. In the appendices, due to obvious problems of space, the necessary notions are simply outlined and they represent more an invitation to make a deeper acquaintance with the subjects than a real effort to provide all the relevant information.

Appendix A on random processes gives an account of the mathematical models on which the techniques of the preceding chapters are based, and as such it should represent the key for their understanding. The subject, however, is too complex to be compressed in a few pages, despite the fact that the principles are very clearly outlined. The same is true for Appendix B on mathematical statistics, and the reader will welcome the bibliographic references which he will need to enlarge his previous knowledge.

The Appendix C on computers gives a short description of how the machines are actually operated. The " on-the-line " averager (the ARC) appears to be so universally useful that every experimenter would probably like to have it at the side of his recording devices.

By reading this book the neurophysiologists will become aware that new and powerful tools applicable to their research have been developed or are in the making. The need for a more complete knowledge of brain functioning is felt more and more in fields of activity outside biology and medicine. Planned industry, applied and social sciences share the largest part of this interest and from them a seizable feed-back of notions, models and suggestions is already reaching the laboratories of the neurophysiologists. The possibilities laid open by these new methodologies for the biologists are certainly worth the effort required for acquiring the background needed for their understanding. We cannot as yet foresee the limitations of these techniques. As for every tool of recent application, these need obviously to be refined and adapted to meet more and more the requirements of the experimentation. As a first approach we need criteria on which " test " of steady states, as applied to nervous activity, can be based. A further extension of this technique, that neurophysiologists will most welcome, is the development of mathematical models describing the change of state of neural activity with time. A large amount of work must be done in cooperation by biologists and physicists: the Communications Biophysics Group of the Massachusetts Institute of Technology deserves the highest praise for the ingenuity of the approach, of which the present book gives a brilliant account.

A. Arduini

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